भारतीय मानक

Indian Standard

IS 548 (Part 1/Sec 2) : 2021

खाद्य तेल और वसा के लिए नमूनाकरण और परीक्षण की विधि

भाग 1 नमूनाकरण, भौतिक और रासायनिक परीक्षण

अनुभाग 2 भौतिक और रासायनिक परीक्षण

Method of Sampling and Test for Oils and Fats

Part 1 Sampling, Physical and Chemical Tests

Section 2 Physical and chemical tests

ICS 67.200.10

भारतीय मानक ब्यूरो

BUREAU OF INDIAN STANDARDS

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Oils and Oilseeds Sectional Committee, FAD 13

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Food and Agriculture Division Council.

IS 548 was first published in 1954 covering the methods of sampling, physical, chemical and qualitative tests for oils and fats. Subsequently, the standard was revised in 1964 and split into 3 parts namely:

Part 1 Methods of sampling, physical and chemical tests

Part 2 Methods for purity tests

Part 3 Methods of analysis of vegetable oils and fats by gas liquid chromatography (GLC) technique

To incorporate latest analytical methods for the additional quality parameters and to make separate standard for sampling, IS 548 (Part 1) 'Method of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests' is being split into two sections:

Section 1 Sampling

Section 2 Physical and chemical tests

This standard prescribes various physical and chemical tests for oils and fats and thus forms a necessary adjunct to the series of Indian Standard specifications for individual oils and fats.

In this section of IS 548 (Part 1), the methods described in IS 548 (Part 1) : 1964 have been refined/updated based on the practical experience of conducting these tests in laboratory and to incorporate latest analytical methods/new test methods such as Karl Fisher method for moisture content, methods for cloud point, diene and triene content, estimation of colour by the Gardner colour scale, anisidine value and alkalinity. IS 548 (Part 1/Sec 1 and 2) would supersede IS 548 (Part 1) : 1964 'Method of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests'.

The composition of the Committee responsible for formulation of the standard is given in Annex A.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD OF SAMPLING AND TEST FOR OILS AND FATS

PART 1 SAMPLING, PHYSICAL AND CHEMICAL TESTS

Section 2 Physical and chemical tests

1 SCOPE

1.1 This standard (Part 1/Sec 2) lays down the methods of test for individual oils, fats, blended oils, fortified oils, fatty materials and co-products. It contains definitions of terms used in trade and industry and prescribes the methods for determination of moisture, insoluble impurities, acid value and free fatty acids, unsaponifiable matter, melting point, refractive index, specific gravity, titre of mixed fatty acids, colour, iodine value (Wijs), saponification value, acetyl value and hydroxyl value, allyl isothiocyanate content, Reichert-Meissl value and Polenske value, cloud point and diene and triene content, anisildine value and alkalinity.

1.2 Should any inconsistency be found to exist between the methods prescribed in this standard and those prescribed in the standard for an individual material, the latter shall prevail.

NOTE — Only calibrated instruments and calibrated glassware shall be used in sampling and testing.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
170 : 2004	Acetone — Specification (*fourth revision*)
250 : 1964	Specification for potassium bichromate, technical and analytical reagent (*revised*)
265 : 1993	Hydrochloric acid — Specification (*fourth revision*)
266 : 1993	Sulphuric acid — Specification (*third revision*)
321 : 1964	Specification for absolute alcohol (*revised*)
323 : 2009	Rectified spirit for industrial use — Specification (*second revision*)
IS 548 (Part 1/Sec 1) : 2021	Method of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests, Section 1 Sampling
1070 : 1992	Reagent grade water — Specification (*third revision*)
1459 : 2018	Kerosene — Specification (*fourth revision*)
1745 : 2018	Petroleum hydrocarbon solvents — Specification (*third revision*)

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Acetyl Value — The number of milligrams of potassium hydroxide required to neutralize the acetic acid liberated by the hydrolysis of one gram of the acetylated oil or fat (*see* **3.3**).

3.1.1 Acetyl value of an oil or fat is a measure of the hydroxyl content of the material.

3.2 Acid Value and Free Fatty Acid — The number of milligrams of potassium hydroxide required to neutralize the free acid present in one gram of the oil or fat under the prescribed conditions.

3.2.1 The acidity of the oil or fat indicated by its acid value is frequently expressed as free fatty acids present in the sample.

3.3 Hydroxyl Value — The number of milligrams of potassium hydroxide required to neutralize the acetic acid capable of combining by acetylation with one gram of the oil or fat (*see also* **3.1**).

3.3.1 Hydroxyl value is equivalent to the hydroxyl content of the material based on the weight of the unacetylated fat.

3.4 Insoluble Impurities — Dirt, meal and other foreign substance which are insoluble in kerosene and petroleum ether under the conditions of the prescribed test.

3.5 Iodine Value (Wijs) — The number of grams of iodine, absorbed per 100 grams of the oil or fat, when determined using Wijs solution.

3.5.1 The iodine value of the oil or fat gives an indication of the degree of unsaturation of the constituent fatty glycerides. Wijs method is applicable to all normal oils and fats not containing conjugated systems.

3.6 Slip Melting Point — The temperature at which the oil or fat softens or becomes sufficiently fluid to slip or run as determined by the open-tube capillary-slip method. In the case of the closed-tube complete-fusion method, it is the temperature at which the oil or fat becomes perfectly clear and liquid.

3.7 Moisture Content — The moisture and any other material contained in the oil or fat which is volatile under the conditions of the prescribed test.

3.8 Polenske Value — The number of millilitres of 0·1 N aqueous sodium hydroxide solution required to neutralize the steam volatile, water insoluble fatty acids distilled from 5 g of an oil or fat under the precise conditions specified in the method.

3.8.1 The Polenske value is the measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids, present in the oil or fat.

3.9 Refractive Index — The ratio of the velocity of light in vacuum to the velocity of light in the oil or fat; more generally, it expressed the ratio between the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of a known wavelength (usually 589·3 nm, the mean of the D lines of sodium) passes from air into the oil or fat.

3.10 Reichert-Meissl value — The number of millilitres of 0·1 N sodium hydroxide solution required to neutralize the steam volatile, water soluble fatty acids distilled from 5 g of an oil or fat under the precise conditions specified in the method.

3.10.1 *Reichert-Meissl Value* — It is a measure of water soluble steam volatile fatty acids, chiefly butyric and caproic acids, present in oil or fat.

3.11 Saponification Value — The number of milligrams of potassium hydroxide required to saponify completely one gram of the oil or fat.

3.12 Specific Gravity

3.12.1 *Specific Gravity of an Oil* — The ratio of the weight in air of a given volume of the oil at 30 °C to the weight in air of an equal volume of water at 30 °C.

3.12.2 *Specific Gravity of a Fat* — The ratio of the weight in air of given volume of the fat at 95 °C to the weight in air of an equal volume of water at 30 °C.

3.13 Titre — The highest temperature attained under standard conditions, during the solidification of the mixed fatty acids obtained from the oil or fat.

3.14 Unsaponifiable Matter — The fraction of substances in oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents.

3.14.1 It includes the higher aliphatic alcohols, sterols, pigments, hydrocarbons and resinous matter.

3.15 Cloud Point — The cloud point is that temperature at which under the conditions of this test a cloud is induced in the sample caused by the first stage of crystallization.

3.16 Anisidine Value — one hundred times the increase in absorbance, measured at a wavelength of 350 nm in a 10 mm cell, of a test solution when reacted with p-anisidine under the test conditions specified in this.

NOTE — The anisidine value has no dimensions, and is calculated and quoted on the basis of 1 g of the test sample in 100 ml of a mixture of solvent and reagent.

3.17 Alkalinity — Proportion of alkali in a fat or oil determined by titration with hydrochloric acid, in accordance with the method specified in this Indian Standard.

4 QUALITY OF REAGENTS

Unless otherwise specified, AR grade chemicals and distilled water (*see* IS 1070) shall be employed in tests.

5 SAMPLING

Representative samples of the material shall be drawn as prescribed in IS 548 (Part 1/Sec 1).

6 DETERMINATION OF MOISTURE CONTENT

6.0 General

Three methods, namely:

a) air-oven method,
b) hot plate method,
c) distillation method, and
d) Karl Fisher method is employed.

The first two methods give the moisture and volatile matter content together while the distillation and Karl Fisher methods give only the water content. The hot-plate method is useful for a rapid preliminary screening.

6.0.1 *Applicability*

The air-oven method is applicable to all the ordinary oils and fats which have a relatively low moisture content (below one percent), but not to drying or semi-drying oils or oils of the coconut oil group. The hot-plate method is applicable to oils and fats with high moisture content. Neither of these two methods is applicable to solvent extracted oils and fats which may contain residues from solvents with fairly high boiling points. The distillation method and Karl Fisher method are applicable to all normal oils and fats, including emulsions, for the determination of moisture only at differentiated from moisture and volatile matter. Distillation method is not applicable to samples of oil or fats containing volatile substances miscible with water.

6.0.2 *Precaution*

Since water tends to settle in samples of oils or fats which have softened or melted, care shall be taken to mix the samples thoroughly so as to distribute the water uniformly. Soften the sample with gentle heat (but do not melt), and mix thoroughly. This general precaution is applicable to all the three methods.

6.0.3 *Referee Method*

In cases or dispute and unless otherwise agreed to between the purchaser and the supplier, the moisture content shall be determined by the Karl Fisher method.

6.1 Air-Oven Method

6.1.1 *Apparatus*

6.1.1.1 *Moisture Dish* — Made of aluminium sheet about 0·45 to 0·56 mm thickness, 70 to 80 mm in diameter and 20 mm deep; provided with tight-fitting slip-over cover.

6.1.1.2 *Desiccator* — Containing an efficient desiccant, such as silica gel, calcium chloride, phosphorus pentoxide.

6.1.1.3 *Air-oven* — Electrically heated, with temperature control device.

6.1.2 *Procedure* — Weigh accurately about 10 g or the oil or fat into a moisture dish which has been dried previously, cooled in the desiccator and then weighed. Place the dish in the air-oven for approximately one hour at 105 ± 1 ºC. Remove the dish from the oven, cool in the desiccator to room temperature and weigh. Repeat this procedure but keep the dish in the oven only for half an hour each time until the difference between the two successive weighing does not exceed one milligram, Preserve the heated oil or fat for the determination of insoluble impurities (*see* **7**).

6.1.3 *Calculation*

Moisture and volatile matter content, percent by weight

$$= \frac{100\ w}{W}$$

where

w = loss in weight in g, of the material upon drying; and

W = weight in g, of the material taken for the test.

6.2 Hot-Plate Method

6.2.1 *Apparatus*

6.2.1.1 *Glass beaker* — 100 to 150 ml capacity.

6.2.1.2 *Small glass rod*

6.2.1.3 *Desiccator* — Containing an efficient desiccant, such as silica gel, calcium chloride, phosphorus pentoxide.

6.2.1.4 *Electric hot-plate* — With variable heat control.

6.2.2 *Procedure*

Weigh accurately about 10 g of the oil or fat into the glass beaker which has been previously dried along with the small glass rod, cooled in the desiccator, and weighed. Heat the sample on the electric hot-plate, stirring continuously with the glass rod. Avoid spattering of the oil or fat which may result from too rapid an ebullition of moisture. The apparent end point is judged by the cessation of the rising bubbles of steam as well as by the absence of foam. *Alternatively,* judge the end point by placing a clean, dry watch-glass on top of the beaker and observing when no further condensation takes place on the watch-glass. When the apparent end point has been reached, heat momentarily to the point of incipient smoking taking care not to overheat. Cool to room temperature in the desiccator and weigh. Preserve the heated oil or fat for the determination of insoluble impurities (*see* **7**).

6.2.3 *Calculation*

Moisture and volatile matter content, percent by weight

$$= \frac{100\ w}{W}$$

where

w = loss in weight in g, of the material upon drying; and

W = weight in g, of the material taken for the test.

6.3 Distillation Method

6.3.1 *Apparatus*

The apparatus consists of a glass flask heated by suitable means and provided with a reflux condenser discharging into a trap and connected to the standard joint flask compatible with the condenser. The trap serves to collect and measure the condensed water, and to return the solvent to the flask. The assembly of the apparatus is shown in Fig. 1, and the various components are described below:

a) *Flask* — 500 ml or 1000 ml flask of the shape shown in Fig. 1, from striae and similar defects.

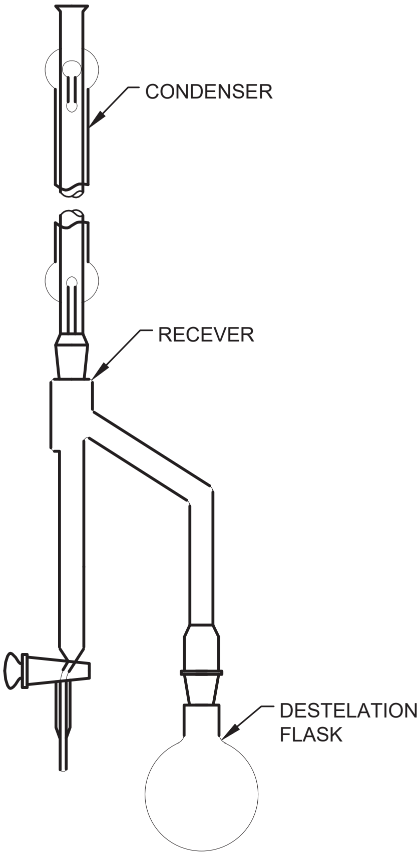
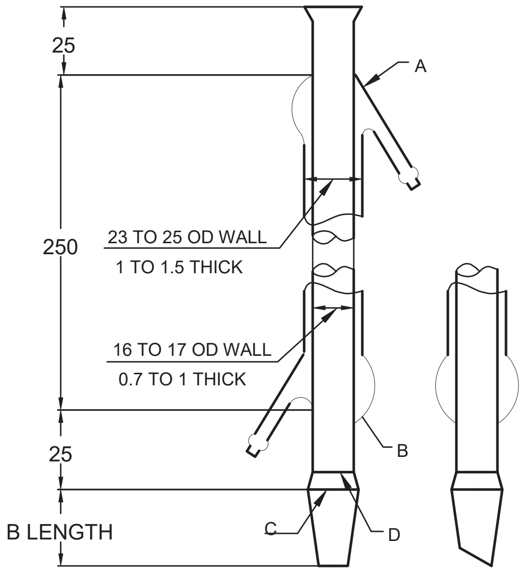


FIG.1 TYPICAL ASSEMBLY OF DEAN AND STARK APPARATUS



All dimensions in millimeters

FIG. 2 CONDENSER

b) *Condenser* — A glass water-cooled reflux type condenser, of the design and dimensions shown in Fig. 2. The only mandatory dimensions for the condenser are the external diameters of the inner tube and of the jacket, which shall be 16 to 17 mm and 23 to 25 mm respectively. The shoulder above the cone of joint *D* shall be elongated, the cone shall be extended beyond the length appropriate to the joint *D,* and the lower end ground at an angle of approximately 60° to the axis. The tip of the condenser shall be 6 to 7 mm above the surface of the liquid in the trap after distillation conditions have been established. The nominal dimensions of the joint *D* are given below:

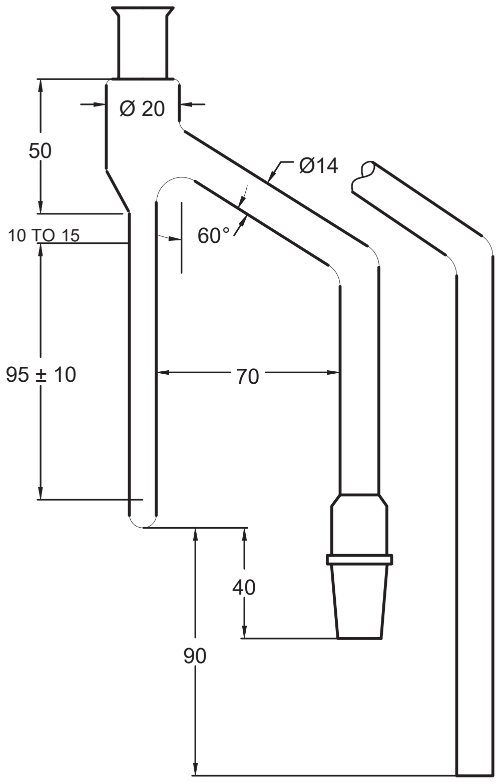
Nominal Dia of large End of Ground Zone	**Nominal Dia of small End of Ground Zone**	**Nominal length of Ground Zone Measured Axially**
mm	mm	mm
18.8	16.2	26

Table 1 Mandatory Dimensions and Tolerances for Receiver

[*Clause* 6.3.1 (c)]

Sl No.	Characteristics	Receiver	
		2 ml	10 ml
(1)	(2)	(3)	(4)
i)	Volume equivalent to smallest sub-division, ml	0.05	0.1
ii)	Scale length, mm	95 ± 10	110 ± 10
iii)	Length of cylindrical tube above upper graduation mark, mm	10 to 15	10 to 30
iv)	Tolerance on capacity, ml	± 0.02	± 0.06
v)	Maximum permissible leakage rate of stopcock, ml/mm	–	0.004

c) *Receivers* (*trap*) — The dimensions and tolerances are given in Fig. 3 and 4; consisting essentially of the upper chamber, together with the tube and ground joint leading to the flask, and the graduated tube.

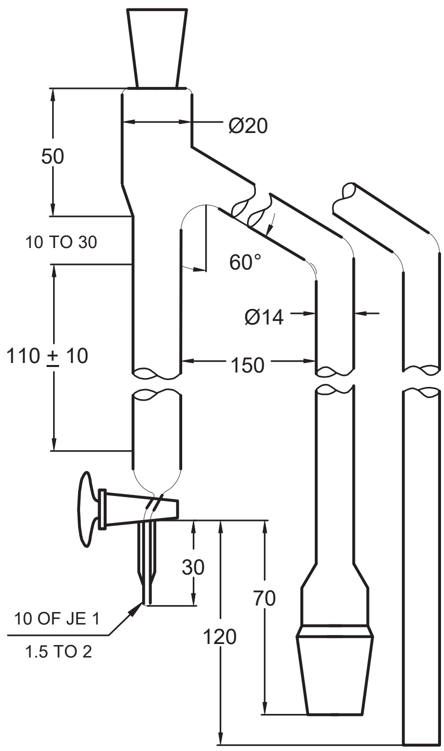


All dimensions in millimeters

FIG. 3 2 ML RECEIVER

The receivers shall be of two sizes, namely, 2 ml capacity and 10 ml capacity (*see* Fig. 3 and 4): The mandatory dimensions and tolerances for the receivers shall be as given in Table 1.

The bottom of the graduated tube of the 2 ml receiver shall be sealed, the end of the tube being approximately hemispherical in shape. The graduated scales on the receivers shall be numbered and subdivided as shown in Fig. 3 and 4. The graduation marks shall be confined to the cylindrical portion of the tube and there shall be no evident irregularity in their spacing.



All dimensions in millimeters

FIG. 4 10 ML RECEIVER

The error at any point on the receiver scale, and also the difference between the errors at any two points on the scale, shall not exceed the figures given for the receivers in Table 1.

For the 10 ml receiver, the stopcock shall be of the 2 mm oblique bore having the general design mown in Fig. 3 and 4.

Each receiver shall have permanently and legibly marked on it:

1) the abbreviation 'ml',
2) the inscription 27 °C to indicate that the receiver is graduated for content at 21 °C, and
3) an identification number shall also appear on the key.

d) *Heat source* — the source of heat may be either an oil-bath or an electric heater provided with a sliding rheostat or other means of heat control. The temperature of the oil in the bath should not be very much higher than the boiling point of xylene or toluene, whichever solvent is used.

e) *Copper wire* — Long enough to extent through the condenser, with one end twisted into a spiral. The diameter of the spiral should be such that it fits snugly within the graduated portion of the receiver and yet may be moved up and down.

6.3.2 *Reagents*

6.3.2.1 *Potassium dichromate-sulphuric acid cleaning solution*

6.3.2.2 *Xylene or toluene*

6.3.3 *Procedure*

Clean the entire apparatus with potassium dichromate-sulphuric acid cleaning solution to minimize the adherence of water droplets to the sides of the condenser and the receiver. Rinse thoroughly with water and dry completely before using. The quantity of material taken for the test is determined by the amount of moisture present (*w/w*).

Moisture Range	Quantity of Material (Approximately)
Less than 1 percent	200 g
1 to 5 percent	100 g
Moisture in excess of 5 percent	proportionally smaller quantity

Place the specified quantity of material accurately weighed, in the distillation flask, add an equal volume of xylene or toluene, as desired, or at least 100 ml if less than 100 g of the material is used, and swirl to mix. Assemble the apparatus and fill the receiver with the solvent by pouring it through the condenser until it begins to overflow into the distillation flask. Insert a loose cotton plug in the top of the condenser to prevent condensation of atmospheric moisture within the tube. In order that the refluxing may be under control, wrap the flask and the tube leading to the receiver with asbestos cloth. Heat the flask so that the distillation rate is about 100 drops per minute, when the greater part of the water has distilled over, increase the distillation rate to about 200 drops per minute and continue until no more water is collected. Purge the reflux condenser occasionally during the distillation with 5 ml portions of xylene or toluene to wash down any moisture adhering to the walls of the condenser. The water in the receiver may be made to separate from the xylene or toluene by moving the spiral copper wire up and down in the condenser and receiver occasionally, thus causing the water to settle at the bottom of the receiver. Reflux until the water-level in the receiver remains unchanged for 30 minutes and then shut off' the source of heat. Flush the condenser with either xylene or toluene, as required, making use of the spiral copper wire to discharge any moisture droplets. Immerse the receiver in water at about 27 °C for at least 15 min or until the xylene or toluene layer is clear, and then read the volume of water.

6.3.4 *Calculation*

$$\text{Moisture content, percent by weight} = \frac{100\,VD}{W}$$

where

V = volume in ml, of water;

D = specific gravity of water at the temperature at which the volume of water is read; and

W = weight in g, of the material taken for the test.

6.4 Karl Fischer Method (Pyridine Free)

6.4.0 This method uses Karl Fischer apparatus and a reagent which is free of pyridine.

6.4.1 *Principle*

Dissolved fat is titrated against an iodine solution and sulfur dioxide (SO_2) is oxidized by iodine in the presence of water. In principle, the chemical reaction in formula given below takes place:

$$H_2O + I_2 + SO_2 + CH_3OH + 3RN \rightarrow [RNH]\,SO_4\,CH_3 + 2\,[RNH]I$$

The alcohol reacts with SO_2 and a nitrogenous base (RN) to form an intermediate alkyl sulfite salt, which is then oxidized by iodine to an alkyl sulfate salt. This oxidation reaction consumes water contained in the sample. The end point is monitored potentiometrically.

6.4.2 *Apparatus*

6.4.2.1 Karl Fischer apparatus, set up according to the manufacturer's recommendations for the determination of water in fats and oils. Set up and conduct protocols for routine maintenance as recommended by the manufacturer. Use an airtight vessel and do not place the instrument in high humidity areas. Do not place instruments or handle samples near water sources, such as taps, sinks, and dishwashers in the laboratory.

6.4.2.2 Analytical balance, readable to the nearest 0.1 mg.

6.4.2.3 Syringes, of capacity 1 ml, 2 ml, 5 ml, 10 ml, and 20 ml.

NOTE — To ensure accurate and reproducible results from the water standard, use a glass gastight syringe. For water standard 10.0, use a 10 ml syringe and for either the water standard 1.00 or 0.10, use a 5 ml syringe. In addition to the appropriate size syringe, use a needle that is long enough to allow for a subsurface injection when injecting through the instrument's septum.

6.4.3 *Reagents*

6.4.3.1 Karl Fischer reagents, consist of one-component reagents or two-component reagents for volumetric determination.

a) One-component reagents, contain all the reactant in the titrant solution: iodine, sulfur dioxide, and imidazole, dissolved in a suitable alcohol. Use a mixture of absolute methanol and absolute chloroform (the methanol content should be at least 25 percent volume fraction, or optimally 50 percent volume fraction).

b) Two-component reagents, consist of all necessary reactants for the titration, but in two different solutions. The titrating agent (usually known as the titrant) contains only iodine and methanol, while the solvent containing the other Karl Fischer reaction components is used as the working medium in the titration cell.

6.4.3.2 Water standard, commercially prepared standard with a certified concentration of 10 mg/g (1.0 percent mass fraction).

6.4.4 *Procedure*

6.4.4.1 *Titre*

The titre shall be determined daily for each bottle of titrant. Prepare the instrument according to the manufacturer's recommendations for calibration. Add 20 ml to 40 ml of working solvent to the titration vessel. The solvent should cover the platinum electrodes. Titrate the vessel to a stable dry end point (take care not to over titrate). Determine the titre of the titrant using the water standard (*see* **6.4.3.4**) and a syringe (*see* **6.4.2.3**). Sample mass is determined by difference. Weigh, to the nearest 0.1 mg, approximately 1 g of the water standard into a syringe, placed on the analytical balance. Upon opening the ampoule, withdraw a small portion of the standard to rinse the syringe; 1 ml to 2 ml is sufficient. Rinse the entire interior of the syringe and discard the rinsings. Then, immediately transfer the remaining standard to the syringe and expel any air bubbles. Using mass by difference make at least three injections from the syringe. When the mass displayed is stable, tare the balance. Inject the water sample into the titration vessel and close the vessel. Place the syringe back on the balance. Record the mass of the water injected to the nearest 0.1 mg. The mass will be displayed as a negative value. Enter the sample mass in the instrument. Start the titration and record the titre when a stable end point is reached. Some instruments may require calculation of titre from the displayed percentage of water. Average a minimum of three titre determinations. Record the arithmetic average. Update the instrument titre value with the new setting.

The titre, $\rho_{titrant}$, in milligrams per millilitre, can be calculated from the formula given below:

$$\rho_{\text{titrant}} = \frac{m_s \, w_{H_2O,s}}{V_{\text{titrant}}}$$

where

m_s = mass, in grams, of the water standard;

$w_{H_2O,s}$ = water content of the certified water standard; and

$V_{titrant}$ = volume, in millilitres, of the titrant used.

NOTE — Titre should not change significantly from day to day with routine operation unless a fresh bottle of titrant has been opened. A problem may exist if more than a 10 percent relative change in titre occurs between days for the same bottle of titrant. If the instrument has been set up more than 12 h before use, the burette should be flushed and the titrant vessel refilled prior to determination of titre. Change the desiccant on the reagent bottles as recommended by the manufacturer to minimize the drift in reagent titre.

6.4.4.2 *Test portion*

By means of a syringe, weigh and introduce a portion of the sample into the instrument using the target masses shown in the Table 2. At least 0.5 ml Karl Fischer reagent shall be used for the titration. Test portion masses are determined by difference. Place the test portion, in an appropriately sized syringe, on the balance. When a stable weight has been achieved, tare balance. Immediately transfer the test portion from the syringe to the reaction vessel.

Place the syringe back on the balance. When the weight is stable, record the mass to the nearest 0.1 mg.

6.4.4.3 *Determination*

Add 20 ml to 40 ml working solvent (*see* **6.4.3.1)** to the titration vessel. The solvent should cover the platinum electrodes. Titrate the vessel to a stable dry end point (take care not to overtitrate). Weigh and introduce the test portion into the instrument according to **6.4.4.2**. Record the moisture content of the test portion when a stable dry end point is reached. Up to six test portions may be assayed before replacing and pre-titrating the working solvent. Replace the working solvent if a precipitate forms indicating that the test portions have not fully dissolved. Pre-titrate the working solvent if there is more than a 10 min delay between running test portions on the same vessel of working solution. The amount of solvent in a two-component solvent system added to the titration vessel determines the maximum

Table 2 Test Portion Sizes for the Volumetric Titration

(*Clause* 6.4.4.2)

Water Content in the Test Portion (Percent mass fraction)	**Min. Mass of Test Portion**[a] (g)	**Water Content in the Test Portion** (mg)
0.001	20	0.2
0.01	20	2
0.1	5	5
1	1	10
5	0.2	10
10	0.1	10
20	0.05	10

[a] The exact test portion mass depends on the precision required and the burette used. However, the test portion mass shall not be greater than the solvent mass. The maximum mass ratio of test portion to solvent is 1 + 1.

amount of water that may be titrated. The volume of solvent or mixed solvent should provide 60 mg to 100 mg of water capacity. The number of test portions that may be titrated before replacing and redrying the working solution depends on sample solubility, reagent capacity of two-component reagent systems, and vessel capacity. The initial volume of working solvent added using the two-component reagents sets a maximum amount of water that can be titrated. The selection of six test portions serves as a guideline but may vary based on water content, reagent titre, and sample solubility.

6.4.5 *Calculation*

Calculations completed automatically by the instrument.

The water content, $w_{H_2O,t}$, in grams per 100 g of test portion, is calculated from the following formula:

$$w_{H_2O,t} = \frac{V_{titrant}\ \rho_{H_2O} \times 100}{m_t \times 1\,000}$$

where

$V_{titrant}$ = volume, in millilitres, of titrant used;

ρ_{H_2O} = titre (water equivalent of the reagent, in milligrams of water per millilitre); and

m_t = mass, in grams, of the test portion.

7 DETERMINATION OF INSOLUBLE IMPURITIES

7.0 General

The material is dissolved in hot kerosene and filtered. The residue is washed thoroughly with petroleum ether dried at 100 ± 2 °C and cooled in a desiccator till constant in weight.

7.1 Apparatus

7.1.1 *Gooch Crucible G3* — Dry to constant weight at 100 ± 2 °C, cool in a desiccator to room temperature and weigh.

7.1.2 *Filler Flask* — of convenient size.

7.1.3 *Gooch Crucible Adaptor*

7.2 Reagents

7.2.1 *Kerosene* — Conforming to Grade 1 of IS 1459, Filter the kerosene through a Gooch crucible prior to use.

7.2.2 *Petroleum Ether* — Conforming to solvent Grade 60-80 of IS 1745.

7.3 Procedure

Use the whole of the heated oil or fat left over after the determination of moisture and volatile matter described under **5.1** or **5.2**. Alternatively, use a sample prepared in the same manner. Add 50 ml or kerosene to this quantity of the oil or fat and heat on a water bath to dissolve it. Filter through the prepared Gooch crucible with the aid of vacuum. Wash the container and the crucible with five 10 ml portions of hot kerosene allowing each portion to drain before adding the next. Wash the crucible thoroughly with petroleum ether to remove all the kerosene. Dry the crucible and contents to constant weight at 100 ± 2 °C, cool to room temperature in a desiccator, and weigh.

7.4 Calculation

$$\text{Insoluble impurities, percent by weight} = \frac{100\,w}{W}$$

where

w = gain in weight in g, of Gooch crucible; and

W = weight in g of the original material taken for the test (*see* **5.1.3** and **5.2.3**).

8 DETERMINATION OF ACID VALUE AND FREE FATTY ACIDS

8.0 General

The acid value is determined by directly titrating the material in an alcoholic medium with aqueous sodium

or potassium hydroxide solution. Free fatty acid is calculated as oleic, lauric, ricinoleic or palmitic acids.

8.1 Reagents

8.1.1 *Ethyl Alcohol* — Ninety-five percent (by volume), or rectified spirit (conforming to IS 323) neutral to phenolphthalein indicator.

8.1.2 *Phenolphthalein Indicator Solution* — Dissolve one gram of phenolphthalein in 100 ml of ethyl alcohol.

NOTE — When testing dark coloured oil or fat, the observation of the end point of the titration may be facilitated either:

a) by using 1percent thymolphthalein in ethanol or alkali blue 6B in place of phenolphthalein, or

b) by adding one millilitre of a 0.1 percent (*w/v*) solution of methylene blue in water to each 100 ml of phenolphthalein indicator solution before the titration.

8.1.3 *Standard Aqueous Potassium Hydroxide or Sodium Hydroxide Solutions* — 0.1 N (higher strength may be used for samples with higher FFA content).

8.2 Procedure

Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity of the cooled oil or fat in a 200 ml conical flask. The weight of the oil or fat taken for the test and the strength of the alkali used for the titration shall be such that the volume of alkali required for the titration does not exceed 10 ml, Add 50 to 100 ml of freshly neutralized hot ethyl alcohol (*see* **8.1.1**) and about one milliliter of phenolphthalein indicator solution. Boil the mixture for about, give minutes and titrate while as hot as possible with standard aqueous alkali solution, shaking vigorously during titration.

8.3 Calculation

$$\text{Acid value} = \frac{56.1\,V\,N}{W}$$

where

v = volume in ml, of standard potassium hydroxide or sodium hydroxide solution used;

N = normality of standard potassium hydroxide or sodium hydroxide solution; and

W = weight in g,of the material taken for the test.

8.3.1 *Free Fatty Acids*

The acidity is frequently expressed as the percentage of free fatty acids present in the sample. The percentage of free fatty acids in most of the oils and fats is calculated on the basis of oleic acid; although in coconut oil and palm kernel oil it is often calculated in terms of lauric acid, in castor oil in terms of ricinoleic acid, and in palm oil in terms of palmitic acid. The calculations in terms of different fatty acids are as follows:

a) Free fatty acids, in terms of oleic acid, percent by weight = $\frac{28.2\,VN}{W}$

b) Free fatty acids, in terms of lauric acid, percent by weight = $\frac{20.0\,VN}{W}$

c) Free fatty acids, in terms of ricinoleic acid, percent by weight = $\frac{29.8\,VN}{W}$

d) Free fatty acids, in terms of palmitic acid, percent by weight = $\frac{25.6\,VN}{W}$

where

v = volume in ml, of standard potassium hydroxide solution used;

N = normality of standard potassium hydroxide solution; and

W = weight in g, of the material taken for the test.

NOTE — Factor will change as per the equivalent weight of fatty acid.

9 DETERMINATION OF UNSAPONIFIABLE MATTER

9.0 General

The material is completely saponified with alcoholic potassium hydroxide solution and extracted with petroleum ether. The petroleum ether extract is washed with aqueous alcohol and then again with water. The washed ether extract is evaporated and the residue is weighed. Unsaponifiable matter is this residue minus the fatty acid present in it, which is determined by titration with sodium hydroxide solution in alcoholic medium.

9.1 Apparatus

9.1.1 *Flat-Bottomed* or *Conical Flask* — 250 ml capacity. An ordinary round, flat-bottomed flask, fitted with a long glass tube which acts as a condenser, may also be used.

9.1.2 *Separating Funnels* — 500 ml capacity.

9.2 Reagent

9.2.1 *Alcoholic Potassium Hydroxide Solution* — Dissolve 70 to 80 g of potassium hydroxide in an equal quantity of distilled water, and add sufficient aldehyde-free ethyl alcohol (95 percent by volume) or aldehyde-free rectified spirit (conforming to IS 323), prepared as described under F-3.4 of IS 323, to make up to 1 000 ml, allow to stand overnight, decant the clear liquid and keep in a bottle closed tightly with a cork or rubber stopper.

9.2.2 *Ethyl Alcohol* — Ninety-five percent (by volume), or rectified spirit (conforming to IS 323).

9.2.3 *Phenolphthalein Indicator Solution* — Dissolve one gram of phenolphthalein in 100 ml of ethyl alcohol.

9.2.4 *Petroleum Ether* — Conforming to solvent Grade 60-80 of IS 1745.

9.2.5 *Aqueous Alcohol* — Containing 10 percent (*v*/*v*) of ethyl alcohol (conforming to IS 321).

9.2.6 *Standard Sodium Hydroxide Solution* — Approximately 0·02 N.

9.2.7 *Acetone* — Free from evaporation residue (*see* IS 170).

9.3 Procedure

9.3.1 Weigh accurately about 5 g of the well-mixed sample into the flask. Add 50 ml of alcoholic potassium hydroxide solution. Boil gently but steadily under a reflux condenser for one hour or until the saponification is complete. Wash the condenser with about 10 ml of ethyl alcohol. Cool the mixture and transfer it to a separating funnel. Complete the transfer by washing the flask first with some ethyl alcohol and then with cold water. Altogether, add 50 ml of water to the separating funnel followed by an addition of 50 ml of petroleum ether. Insert the stopper and shake vigorously for at least one minute and allow to settle until both the layers are clear. Transfer the lower layer containing the soap solution to another separating funnel, and repeat the ether extraction at least six times more using 50 ml of petroleum ether for each extraction. If any emulsion is formed, add a small quantity of ethyl alcohol or alcoholic potassium hydroxide solution.

9.3.2 Collect all the ether extracts in a separating funnel. Wash the combined extracts in the funnel three times with 25 ml portions of aqueous alcohol shaking vigorously and drawing off the alcohol-water layer after each washing. Again wash the ether layer successively with 20 ml portions of water until the wash-water no longer turns pink on addition of a few drops of phenolphthalein indicator solution. Do not remove any of the ether layers. Transfer the ether layer to a tared flask containing a few pieces of pumice stone, and evaporate to dryness on a water-bath under a gentle stream of clean dry air. To remove the last traces of ether, place the flask in an air-oven at 80 to 90 °C for about one hour. To remove the last traces of moisture, add a few millilitres of acetone and pass a gentle stream of clean dry air over the surface of the material or evacuate using a water vacuum pump at about 50 ºC for about 15 min. Cool in a desiccator and weigh. Repeat the evacuating, cooling and weighing until a constant weight is-obtained.

9.3.3 After weighing, take up the residue in 50 ml of warm neutral ethyl alcohol, containing a few drops of phenolphthalein indicator solution and titrate with standard sodium hydroxide solution.

9.4 Calculation

9.4.1 Weight in g or the fatty acids in the extract (as oleic acid) = $B = 0.282\ VN$.

where

V = volume in ml, of standard sodium hydroxide solution; and

N = normality of standard sodium hydroxide solution.

9.4.2 Unsaponifiable matter, percent by weight

$$= \frac{100\,(\mathrm{A} - B)}{W}$$

where

A = Weight in g, of the residue (*see* 8.3.2);

B = weight in g, of the fatty acids in the extract (*see* **8.4.1**); and

W = weight in g, of the material taken for the test.

10 DETERMINATION OF SLIP MELTING POINT

10.0 General

Oils and fats are chiefly mixtures of glycerides. They do not exhibit either a definite or a sharp melting point. Therefore, the term 'melting point' does not imply the same characteristics that it does with pure crystalline substances. Fats pass through a stage of gradual softening before they become completely liquid. The melting point is, therefore, defined by the specific conditions of the method by which it is determined (*see* **3.6**).

10.0.1 The melting point is determined by taking the solid fat inside a small capillary tube and two methods are prescribed for the purpose. Both of these are applicable to all types of normal animal and vegetable fats and the method used shall be specified while stating the results.

10.1 Open-Tube Capillary-slip Method

10.1.1 *Apparatus*

10.1.1.1 *Melting point tubes* — Thin walled, uniformly bored capillary glass tubes open at both ends and with the following dimensions:

a) Length, 50 to 60 mm;

b) Inside diameter 0.8 to 1.1 mm; and

c) Outside diameter, 1.2 to 1.5 mm.

10.1.1.2 *Thermometer* — With suitable temperature range (preferably –10 to 110 °C with least count of 0.1 °C).

10.1.1.3 *Beaker* — With a side-tube heating arrangement. Thiele melting point tube may be used.

10.1.1.4 *Suitable heating source*

10.1.2 *Procedure*

Melt the sample and filter it through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is absolutely dry. Mix the sample thoroughly. Insert a clean melting point tube into the molten sample product so that a column of the material, about 10 mm long, is forced into the tube. Chill the sample in the tube at once by placing the end of the tube containing the sample against a piece of the ice until the fat has solidified. Place the melting point tube in a test-tube and hold it for one hour either in a refrigerator or in water maintained at 4 to 10 ºC. Remove the melting point tube and attach with a rubber band or any other suitable means to the thermometer 10 that the lower end of the melting point tube is even with the bottom of the bulb of the thermometer. Pour water at about 10 ºC into the beaker or the Thiele tube, and suspend the thermometer in the centre of the apparatus, so that the lower end or the sample column is about 30 mm below the surface of water. Heat the side tube of the apparatus gently, so that the temperature of the water rises slowly at the rate of 2 °C per minute till the temperature reaches 25 °C, and thereafter at the rate of 0.5 ºC per minute. Note the temperature of the water when the sample column commences to rise in the melting point tube. Report the average of two such separate determinations as the melting point, provided that the readings do not differ by more than 0.5 °C.

10.2 Closed-Tube Complete-Fusion Method

10.2.1 *Apparatus*

10.2.1.1 *Melting Point Tubes* — Thin-walled, uniformly bored capillary glass tubes open at both ends and with the following dimensions:

a) Length, 50 to 60 mm;
b) Inside diameter, 0.8 to 1.1 mm; and
c) Outside diameter, 1.2 to 1.5 mm.

10.2.1.2 *Thermometer* — 0.1º subdivisions and a suitable range.

10.2.13 *Large Test-tube*

10.2.14 *Glass Beaker* — 600 ml capacity.

10.2.1.5 *Suitable heat source*

10.2.2 *Procedure*

Melt the sample and filter it through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is absolutely dry. Mix the sample thoroughly. Insert a clean melting point tube into the molten product so that a column of the material about 10 mm long is forced into the tube. Cautiously fuse one end of the tube (where the sample is located) in a small flame, taking care not to burn the fat. Place the tube in beaker and while the fat is still in the liquid state, transfer to refrigerator and hold at 4 to 10 °C overnight (about 16 h). Remove the tube from the refrigerator, and attach with a rubber band or by any other suitable means to the thermometer so that the lower end of the melting point tube is at the same level as the bottom of the bulb of the thermometer. Suspend the thermometer in a large test-tube containing water and immerse it in the 600 ml beaker which is about half full of water. The bottom of the thermometer is immersed in the water about 30 mm below the surface. Adjust the starting bath temperature from 8 to 10 ºC below the melting point of the sample at the beginning of the test. Agitate the water in the large test-tube as well as in the beaker with a small stream of air or by other means, and apply heat so u to increase the bath temperature at the rate of about 0.5 ºC per minute. As fats usually pass through an opalescent stage before melting completely. Heating is continued until the liquid in the tube is completely clear throughout. Observe the temperature at which the liquid becomes clear. Report the average of two such separate determinations as the melting point, provided that the readings do not differ by more than 0·5 °C.

Report the average of two such separate determinations as the melting point, provided that the readings do not differ by more than 0·5 °C.

11 DETERMINATION OF REFRACTIVE INDEX

11.1 Apparatus

11.1.1 *Refractometer* — Abbe or Butyro refractometer. The temperature of the refractometer should be controlled to within ±0.1 °C and for this purpose it should be provided with a thermostatically controlled water-bath and a motor-driven pump to circulate water through the instrument. The instrument should be standardized, following the manufacturer's instructions, with a liquid of known purity and refractive index or with a glass prism of known refractive index. Distilled water, which has a refractive index of 1.3330 at 20.0 °C, is a satisfactory liquid for standardization.

11.1.2 *Light Source* — If the refractometer is equipped with a compensator, a tungsten lamp or a daylight bulb may be used. Otherwise, a monochromatic light, such as an electric sodium vapour lamp, should be used.

11.2 Procedure

Melt the sample, if it is not already liquid, and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample

is completely dry. Adjust the temperature of the refractometer to 40±0.1 °C or to any other desired temperature. Ensure that the prisms are clean and completely dry, and then place a few drops of the sample on the lower prism. Close the prisms, tighten firmly with the screw-head, and allow to stand for one or two minutes. Adjust the instrument and light to obtain the most distinct reading possible, and determine the refractive index.

11.3 Temperature Correction

Unless the correction factors are specified in the detailed specification, approximate corrections shall be made using the following equation:

$$R = R' + K\,(T' - T)$$

where

R = the reading of the refractometer reduced to the specified temperature, T ºC;

R' = the reading at T' ºC;

K = constant, 0.000 365 for fats, and 0·000 385 for oils (*if Abbe refractometer is used*), or 0.55 for fats and 0.58 for oils (*if Butyro refractometer is used*); and

T' = the temperature at which the reading R' is taken; and

T = the specified temperature (generally 40.0 °C).

11.4 Conversion of Butyro Refractometer Reading to Refractive Indices

When Butyro refractometer is used, its readings shall be converted into refractive indices (n_D) using Table 3.

Table 3 Conversion of Butyro Refractometer Readings (B) to Refractive Indices (n_D)

(*Clause* 11.4)

B	n_D	B	n_D	B	n_D	B	n_D
0.0	1.4220	21.7	1.4390	45.2	1.4560	71.1	1.4730
0.1	1	21.8	1	45.3	1	71.3	1
0.2	2	22.0	2	45.5	2	71.4	2
0.4	3	22.1	3	45.6	3	71.6	3
0.5	4	22.2	4	45.7	4	71.8	4
0.6	5	22.4	5	45.9	5	71.9	5
0.7	6	22.5	6	46.0	6	72.1	6
0.9	7	22.6	7	46.2	7	72.2	7
1.0	8	22.7	8	46.3	8	72.4	8
1.1	9	22.9	9	46.4	9	72.5	9
1.2	1.4230	23.0	1.4400	46.6	1.4570	72.7	1.4740
1.4	1	23.2	1	46.7	1	72.9	1
1.5	2	23.3	2	46.9	2	73.0	2
1.6	3	23.4	3	47.0	3	73.2	3
1.7	4	23.5	4	47.2	4	73.3	4
1.9	5	23.7	5	47.3	5	73.5	5
2.0	6	23.8	6	47.5	6	73.7	6
2.1	7	23.9	7	47.6	7	73.8	7
2.2	8	24.1	8	47.7	8	74.0	8
2.4	9	24.2	9	47.9	9	74.1	9
2.5	1.4240	24.3	1.4410	48.0	1.4580	74.3	1.4750
2.6	1	24.5	1	48.2	1	74.5	1
2.7	2	24.6	2	48.3	2	74.6	2
2.8	3	24.7	3	48.5	3	74.8	3
3.0	4	24.8	4	48.6	4	75.0	4
3.1	5	25.0	5	48.8	5	75.1	5
3.2	6	25.1	6	48.9	6	75.3	6
3.3	7	25.2	7	49.1	7	75.5	7
3.5	8	25.4	8	49.2	8	75.6	8
3.6	9	25.5	9	49.4	9	75.8	9
3.7	1.4250	25.6	1.4420	49.5	1.4590	76.0	1.4760
3.8	1	25.8	1	49.7	1	76.1	1
4.0	2	25.9	2	49.8	2	76.3	2
4.1	3	26.1	3	50.0	3	76.5	3
4.2	4	26.2	4	50.1	4	76.7	4
4.3	5	26.3	5	50.2	5	76.8	5
4.5	6	26.5	6	50.4	6	77.0	6
4.6	7	26.6	7	50.5	7	77.2	7
4.7	8	26.7	8	50.7	8	77.3	8
4.8	9	26.9	9	50.8	9	77.5	9
5.0	1.4260	27.0	1.4430	51.0	1.4600	77.7	1.4770
5.1	1	27.1	1	51.1	1	77.9	1
5.2	2	27.3	2	51.3	2	78.1	2
5.4	3	27.4	3	51.4	3	78.2	3
5.5	4	27.5	4	51.6	4	78.4	4
5.6	5	27.7	5	51.7	5	78.6	5
5.7	6	27.8	6	51.9	6	78.7	6
5.9	7	27.9	7	52.0	7	78.9	7
6.0	8	28.1	8	52.2	8	79.1	8
6.1	9	28.2	9	52.3	9	79.2	9
6.2	1.4270	28.3	1.4440	52.5	1.4610	79.4	1.4780
6.4	1	28.5	1	52.7	1	79.6	1
6.5	2	28.6	2	52.8	2	79.8	2
6.6	3	28.7	3	53.0	3	80.0	3
6.8	4	28.9	4	53.1	4	80.1	4
6.9	5	29.0	5	53.3	5	80.3	5
7.0	6	29.2	6	53.4	6	80.5	6
7.1	7	29.3	7	53.6	7	80.6	7
7.2	8	29.4	8	53.7	8	80.8	8
7.4	9	29.6	9	53.9	9	81.0	9
7.5	1.4280	29.7	1.4450	54.0	1.4620	81.2	1.4790
7.6	1	29.9	1	54.2	1	81.3	1
7.7	2	30.0	2	54.3	2	81.5	2
7.9	3	30.1	3	54.5	3	81.7	3

Table 3 (*Continued*)

B	n_D	B	n_D	B	n_D	B	n_D
8.0	4	30.3	4	54.6	4	81.9	4
8.1	5	30.4	5	54.8	5	82.0	5
8.2	6	30.6	6	55.0	6	82.2	6
8.4	7	30.7	7	55.1	7	82.4	7
8.5	8	30.8	8	55.3	8	82.5	8
8.6	9	30.9	9	55.4	9	82.7	9
8.7	1.4290	31.0	1.4460	55.6	1.4630	82.9	1.4800
8.9	1	31.2	1	55.7	1	83.1	1
9.0	2	31.4	2	55.9	2	83.2	2
9.1	3	31.5	3	56.0	3	83.4	3
9.2	4	31.6	4	56.2	4	83.6	4
9.4	5	31.8	5	56.3	5	83.8	5
9.5	6	31.9	6	56.5	6	83.9	6
9.6	7	32.1	7	56.6	7	84.1	7
9.8	8	32.2	8	56.8	8	84.3	8
9.9	9	32.3	9	56.9	9	84.5	9
10.0	1.4300	32.5	1.4470	57.1	1.4640	84.6	1.4810
10.1	1	32.6	1	57.3	1	84.8	1
10.3	2	32.8	2	57.4	2	85.0	2
10.4	3	32.9	3	57.6	3	85.2	3
10.5	4	33.0	4	57.7	4	85.3	4
10.6	5	33.2	5	57.9	5	85.5	5
10.7	6	33.3	6	58.0	6	85.7	6
10.9	7	33.5	7	58.2	7	85.9	7
11.0	8	33.6	8	58.3	8	86.0	8
11.1	9	33.7	9	58.5	9	86.2	9
11.3	1.4310	33.9	1.4480	58.6	1.4650	86.4	1.4820
11.4	1	34.0	1	58.8	1	86.6	1
11.5	2	34.2	2	58.9	2	86.7	2
11.6	3	34.3	3	59.1	3	86.9	3
11.8	4	34.4	4	59.2	4	87.1	4
11.9	5	34.6	5	59.4	5	87.3	5
12.0	6	34.7	6	59.5	6	87.5	6
12.2	7	34.9	7	59.7	7	87.6	7
12.3	8	35.0	8	59.8	8	87.8	8
12.4	9	35.1	9	60.0	9	88.0	9
12.5	1.4320	35.3	1.4490	60.2	1.4660	88.2	1.4830
12.7	1	35.5	1	60.3	1	88.3	1
12.8	2	35.6	2	60.5	2	88.5	2
12.9	3	35.7	3	60.6	3	88.7	3
13.0	4	35.8	4	60.8	4	88.9	4
13.2	5	36.0	5	60.9	5	89.1	5
13.3	6	36.1	6	61.1	6	89.2	6
13.5	7	36.3	7	61.2	7	89.4	7
13.6	8	36.4	8	61.4	8	89.6	8
13.7	9	36.5	9	61.5	9	89.8	9
13.8	1.4330	36.7	1.4500	61.7	1.4670	90.0	1.4840

B	n_D	B	n_D	B	n_D	B	n_D
14.0	1	36.8	1	61.8	1	90.2	1
14.1	2	37.0	2	62.0	2	90.3	2
14.2	3	37.1	3	62.2	3	90.5	3
14.4	4	37.2	4	62.4	4	90.7	4
14.5	5	37.4	5	62.5	5	90.9	5
14.6	6	37.5	6	62.6	6	91.1	6
14.7	7	37.7	7	62.8	7	91.2	7
14.9	8	37.8	8	62.9	8	91.4	8
15.0	9	37.9	9	63.1	9	91.6	9
15.1	1.4340	38.1	1.4510	63.2	1.4680	91.8	1.4850
15.3	1	38.2	1	63.4	1	92.0	1
15.4	2	38.3	2	63.5	2	92.1	2
15.5	3	38.5	3	63.7	3	92.3	3
15.6	4	38.6	4	63.8	4	92.5	4
15.8	5	38.7	5	64.0	5	92.7	5
15.9	6	38.9	6	64.2	6	92.9	6
16.0	7	39.0	7	64.3	7	93.0	7
16.2	8	39.2	8	64.5	8	93.2	8
16.3	9	39.3	9	64.7	9	93.4	9
16.4	1.4350	39.5	1.4520	64.8	1.4690	93.6	1.4860
16.6	1	39.6	1	65.0	1	93.8	1
16.7	2	39.7	2	65.1	2	94.0	2
16.8	3	39.9	3	65.3	3	94.1	3
17.0	4	40.0	4	65.4	4	94.3	4
17.1	5	40.1	5	65.6	5	94.5	5
17.2	6	40.3	6	65.7	6	94.7	6
17.4	7	40.4	7	65.9	7	94.8	7
17.5	8	40.6	8	66.1	8	95.0	8
17.6	9	40.7	9	66.2	9	95.2	9
17.8	1.4360	40.9	1.4530	66.4	1.4700	95.4	1.4870
17.9	1	41.0	1	66.5	1	95.6	1
18.0	2	41.1	2	66.7	2	95.8	2
18.2	3	41.3	3	66.8	3	96.0	3
18.3	4	41.4	4	67.0	4	96.1	4
18.4	5	41.5	5	67.2	5	96.3	5
18.5	6	41.7	6	67.3	6	96.5	6
18.7	7	41.8	7	67.5	7	96.7	7
18.8	8	42.0	8	67.7	8	96.9	8
18.9	9	42.1	9	67.8	9	97.0	9
19.1	1.4370	42.3	1.4540	68.0	1.4710	97.2	1.4880
19.2	1	42.4	1	68.1	1	97.4	1
19.3	2	42.5	2	68.3	2	97.6	2
19.5	3	42.7	3	68.4	3	97.8	3
19.6	4	42.8	4	68.6	4	98.0	4
19.7	5	43.0	5	68.7	5	98.1	5
19.8	6	43.1	6	68.9	6	98.3	6
20.0	7	43.3	7	69.1	7	98.5	7

Table 3 (*Concluded*)

B	n_D	B	n_D	B	n_D	B	n_D	B	n_D	B	n_D	B	n_D	B	n_D
20.1	8	43.4	8	69.2	8	98.7	8	20.9	4	44.3	4	70.2	4	99.8	4
20.3	9	43.6	9	69.4	9	98.9	9	21.1	5	44.4	5	70.3	5	100.0	1.4895
20.4	1.4380	43.7	1.4550	69.5	1.4720	99.1	1.4890	21.2	6	44.6	6	70.5	6		
20.5	1	43.9	1	69.7	1	99.2	1	21.3	7	44.7	7	70.7	7		
20.6	2	44.0	2	69.9	2	99.4	2	21.4	8	44.9	8	70.8	8		
20.8	3	44.2	3	70.0	3	99.6	3	21.6	9	45.0	9	71.0	9		

12 DETERMINATION OF SPECIFIC GRAVITY

12.0 General

The specific gravity may be determined with a Westphal hydrostatic balance, or with a specific gravity bottle or pyknometer. The latter method shall be adopted as the referee method in cases of dispute. The temperatures at which the specific gravity is determined shall be reported, namely, sp gr 30 °C/30 °C or sp gr 95 °C/30 °C.

12.1 Preparation of the Material

Melt the sample, if necessary, and filter through a filter paper to remove any impurities and the last traces of moisture, make sure that the sample is completely dry. Cool the sample to 30 °C or warm to the desired test temperature.

12.2 Westphal Hydrostatic Balance Method

Suspend the plummet in the cylinder filled with recently boiled distilled water at the test temperature, and place the largest rider on the hook. Adjust the screw on the base until the pointer is exactly opposite the fixed indicator point. Wipe the plummet and the cylinder to remove the water. Fill the cylinder with the material at the lame temperature and dip the plummet into the material, removing air bubbles, if any, formed in the eyehole of the plummet, by lifting it from the material. Re-immerse the plummet in the material and adjust the height of the balance to ensure that the plummet will be nearly in the middle of the material when the beam is counterpoised. Place riders on the beam, till the pointer and the fixed indicator are exactly opposite each other. Read the specific gravity from the position of the riders on the beam, beginning with the largest and ending with the smallest.

12.2.1 *Corrections*

Sometimes, especially when using the hydrostatic balance, it is not convenient to make the determination at the specified temperature. The determination may be made at a convenient temperature (T’) as near to the specified temperature (T) as possible and the result corrected as shown below to the specified temperature. This correction is based on the average value for the coefficient of expansion of oils and fats (0·000 64 per 1 °C) and for water (0·000 23 per 1 °C). Approximate temperature corrections may, therefore, be made as follows:

a) Specific gravity at T ºC/T ºC =

$$\frac{D' + 0.000\ 64\ (T'-T)}{W' + 0.000\ 23\ (T'-T)}$$

where

D' = density of oil fat at T ºC;

T' = the temperature at which the densities D' and W' were determined;

T = the standard temperature, 30 °C; and

W' = density of water at T ’°C.

b) Specific gravity at T ºC/T ºC = $S' + 0{\cdot}000\ 41\ (T' - T)$

where

S' = Specific gravity at T'/T ºC;

T' = temperature at which the specific gravity was determined; and

T = standard temperature, 30 ºC.

12.3 Specific Gravity Bottle or Pyknometer Method

12.3.1 *Apparatus*

12.3.1.1 *Specific Gravity Bottle or Pyknometer* — Clean and dry the bottle or pyknometer thoroughly, weigh and then fill with recently boiled and cooled water at about 25 °C after removing the cap of the side arm. Fill to overflowing by holding the bottle or pyknometer on its side in such a manner as to prevent the entrapment of air bubbles. Insert the stopper and immerse in a water-bath at the desired test temperature ± 0·2 °C. Keep the entire bulb completely covered with water and hold at that temperature for 30 min. Carefully remove any water which has exuded from the capillary opening. Remove from the bath, wipe completely dry, replace the cap, cool to room temperature and weigh, calculate the weight of water. This is a constant for the bottle or pyknometer, but should be checked periodically. A specific gravity bottle of about 50 ml capacity and of either of the two shapes as shown in Fig. 5 is recommended.

12.3.1.2 *Water bath* — Maintained at 30±0.1 °C, or 95±0.1 °C, as required.

12.3.1.3 *Thermometer* — Of a suitable range, with 0.1 °C subdivisions.

12.3.2 *Procedure*

Fill the bottle with the oil previously cooled to about 25 °C or the melted fat to overflowing, holding the bottle on its side in such a manner as to prevent the entrapment of air bubbles after removing the cap of the side arm. Insert the stopper, immerse in the water-bath at 30±0·2 °C, or at 95±0·2 °C, as required, and hold for 30 min. Carefully wipe off any oil which has come through the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Replace the cap of tile side arm, cool to room temperature and weigh.

12.3.3 *Calculation*

a) Specific gravity at 30 °C/30 °C = $\frac{A-B}{C-B}$

where

A = weight in g, of the specific gravity bottle with oil at 30 °C;

B = weight in g, of the specific gravity bottle; and

C = weight in g, of the specific gravity bottle with water at 30 °C.

b) Specific gravity $A-B$ at 95 °C/30 °C = $(C-B) \times [1 + (0{\cdot}000025 \times 65)]$

where

A = weight in g, of the specific gravity bottle and fat at 95 °C;

B = weight in g, of the specific gravity bottle;

C = weight in g, of the specific gravity bottle with water at 30 °C; and 0·000 025 = coefficient of expansion of glass.

12.3.4 *Corrections — see* 12.2.1.

NOTE — Method A shall be used as a referee method whereas Method B may be used for routine analysis.

13 TITRE TEST

13.0 General

The fatty oil is saponified with glycerol-caustic potash solution, and the soap is acidified with dilute sulphuric acid to give fatty acids. The fatty acids obtained are washed and dried. The highest temperature recorded during the solidification under standard conditions is the titre.

13.1 Apparatus

The assembly of the apparatus is shown in Fig. 6, and the various components are described under **13.1.2** to **13.1.8**.

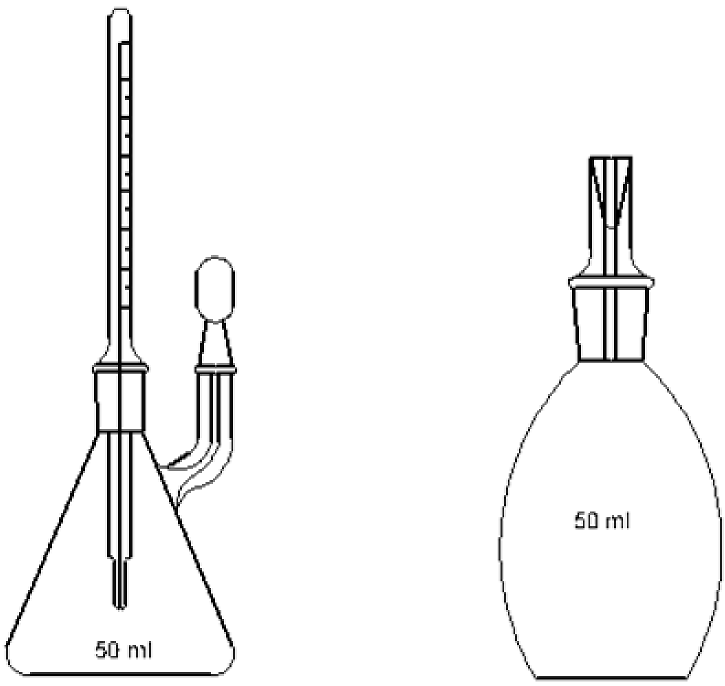


FIG. 5 SPECIFIC GRAVITY BOTTLES

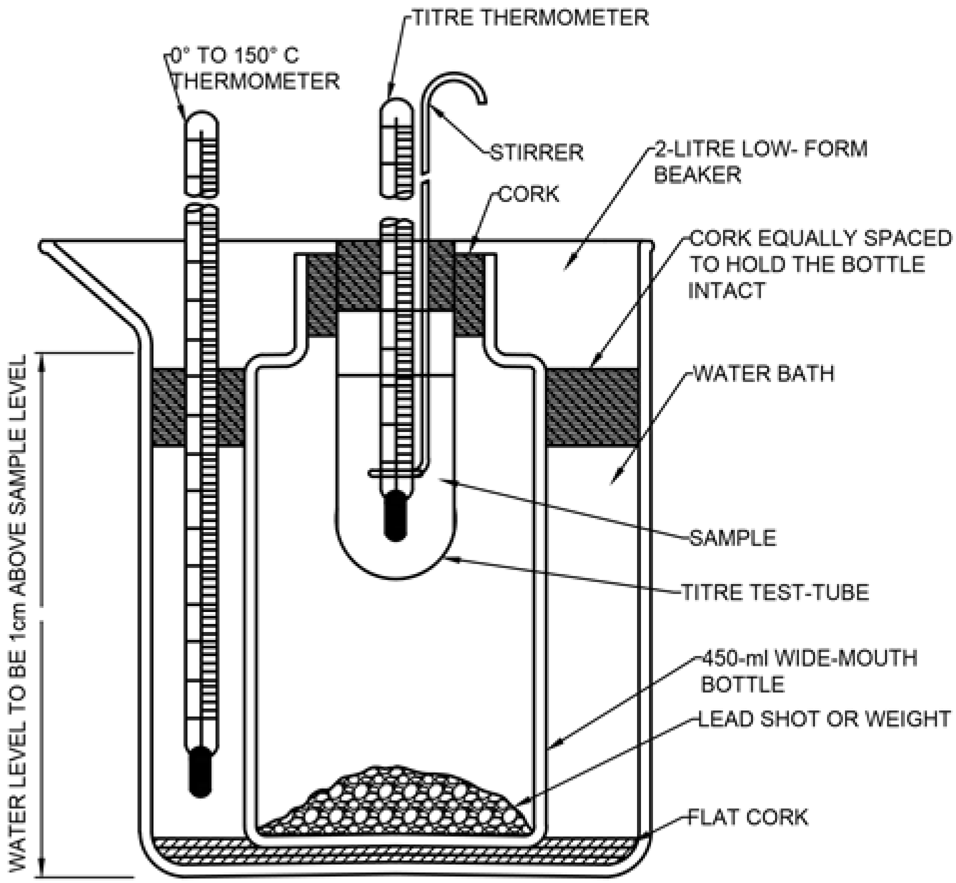


FIG. 6 ASSEMBLY OF APPARATUS FOR TITRE TEST

13.1.1 *Saponification Flask* — 250 or 500 ml capacity.

13.1.2 *Beaker* — 2 litre capacity, to serve as a water-bath.

13.1.3 *Wide-Mouth Bottle* — 450 ml capacity, height 190 mm and inside diameter neck 38 mm.

13.1.4 *Titre Test-Tube* — About 100 mm in length, 25 mm in diameter and one millimetre wall thickness, with an etched mark extending around the tube at a distance of approximately 55 mm from the bottom to show the height to which the tube is to be filled.

13.1.5 *Stirrer* Made of glass, stainless steel, with one end bent in the form of a loop of 19 mm outside diameter. The upper end may be: formed to suit stirring with hand or attached to a mechanical stirrer.

13.1.6 *Laboratory Thermometer* — Range 0 to 110 °C.

13.1.7 *Corks* — As shown in Fig. 6.

13.1.8 *Titre Thermometer* — with the following characteristics:

a) *Type*-etched stem glass.
b) *Liquid*-mercury.
c) *Filling above liquid*-evacuated or nitrogen gas.
d) *Thermal range* – minus – 10 to – 50 °C.
e) *Subdivisions* – 0.1 or 0.2 °C.
f) *Distance from bottom of bulb to minus 10* °C *mark* – 50 to 60 mm.
g) *Distance from 50* °C mark to top of thermometer – 20 to 35 mm.
h) *Immersion* – 45 mm, A line shall be etched around the stem 45 mm from the bottom of the bulb.
j) Maximum scale error permitted at any point – *0.2 °C.*
k) *Standardization* - The thermometer shall be standardized at the melting point of ice and at intervals of approximately 20 °C, for the condition of 45 mm immersion and for an average stem temperature of the emergent mercury column of 25 °C.

13.2 Reagents

13.2.1 *Glycerol-Caustic Potash Solution* — Dissolve with the aid of heat, 250 g of solid potassium hydroxide in 1250 g of glycerine. Do not heat above 140 °C.

13.2.2 *Dilute Sulphuric Acid* — 30 percent by weight obtained by cautiously adding 16 ml of concentrated sulphuric acid, sp gr 1·84 (conforming to IS 266) to 70 mm of water.

13.2.3 *Methyl Orange Indicator Solution* — Dissolve 0.1 g of methyl orange in 100 ml of water.

13.2.4 *Acetone* — Conforming to IS 170.

13.3 Procedure

13.3.1 *Preparation of Fatty Acids*

Weigh about 70 g of the glycerol caustic potash solution into the saponification flask. Heat to 150 °C while stirring. Add about 30 g of the sample of oil or melted fat and re-heat to about 150 °C. If necessary, add a little more glycerol-caustic potash solution to ensure complete saponification (complete saponification is usually indicated by an initial change in the appearance of the mass often accompanied by an increase in the viscosity or thickening. The solution then thins out after the reaction is complete and assumes a homogeneous appearance. The most common characteristic is that of soap bubbles forming and rising from the surface. Considerable care should be exercised at all times to ensure complete saponification). Cool lightly and dissolve the soap in 300 ml of water contained in a 1 000 ml beaker. Add dilute sulphuric acid until the solution is distinctly acidic to methyl orange indicator, and place the beaker in a boiling water-bath until the fatty acids correct as a clear layer at the top. Siphon off the lower aqueous acid layer, add 300 ml of hot water, place in the boiling water-bath for a few minutes, and again siphon off the aqueous acid layer. Wash the fatty, acids thrice in this manner or until the last traces of soap and acid are removed. The acidification and washing should be done in as short a period as possible, keeping the beaker covered to prevent the oxidation of the fatty acids. After the last wash, allow the fatty acids to settle for a few minutes and then decant them carefully. Filter through one or two thicknesses of filter paper introduced into a conical flask, and add about 10 ml of acetone. Close the flask with an air-tight cork, carrying a glass tube. Immerse the flask in boiling water and apply suction from a water pump until all bubbling ceases. Remove the cork and dry the contents of the flask at about 105 °C for at least half an hour.

13.3.2 *Determination of Titre*

Fill the low-form beaker with water up to two-thirds of its capacity. Adjust the temperature of water between 15 °C and 20 °C below the expected titre point when it is not above 35 °C, and at 20 ± 1 °C when it is 35 °C or higher. Fill the test-tube up to the mark with the fatty acid preparation described in **13.3.1**, at a temperature 10 to 12 °C higher than the expected titre point. Insert the titre thermometer in the centre of the sample and adjust its height so that its immersion mark coincides with the top surface of the fatty acid layer. When the temperature of the fatty acid comes down to about 10 °C higher than the titre point, set the stirrer moving in a vertical direction at a rate of about 60 complete up and down motions per minute, The temperature of the fatty acid gradually, comes down and stirring is continued until the temperature remains constant for 30 s. The stirring is stopped when the temperature begins to rise and the stirrer is raised out of the sample. The highest temperature recorded by the thermometer during this rise is the titre point. Duplicate determinations should agree within 0.2 °C.

14 DETERMINATION OF COLOUR

14.1 Lovibond Method

This method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond tintometer.

14.1.1 *Apparatus*

14.1.1.1 *Lovibond tintometer or colorimeter*

14.1.1.2 *Glass Cells* — Presently, the Lovibond cell designations generally employed in the laboratories in the country, namely, ¼ in, ½ in, 1 in and 5 ¼ in are recommended, However, with the corresponding metric designations of the cells, such as 1, 2, 5 and 10 cm, also becoming available, their use will involve reconsideration of the requirements for colour of oils and fats.

14.1.1.3 *Filter paper*

14.1.2 *Procedure*

Melt the sample, if it is not already liquid, and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is absolutely clear and free from turbidity. Clean the glass cell of the desired size (*see* **14.1.3.2**) with chloroform and allow it to dry. Fill it with the clear filtered sample and place the cell in position in the tintometer, Place along side of it such red, yellow, blue or neutral Lovibond glass slides or any, combinations of these as are necessary to match the colour shade of the oil, observing the colours of the oil and of the combination of the glass slides through an eyepiece.

14.1.3 *Report*

Report the colour of the oil in terms of Lovibond units as follows:

Colour reading in (*) cell

$$= (a\ \mathrm{Y} + 5\ b\ \mathrm{R}) \text{ or } (a\ \mathrm{Y} + 10\ b\ \mathrm{R})$$

where

a = the sum total of the various yellow (Y) slides used; and

b = the sum total of the various red (R) slides used.

**Size designation of cell used.*

14.1.3.1 Although the yellow and red slides required to match the colour shade of an oil in a Lovibond tintometer are assessed separately, it is found that to a certain extent these slides arc mutually compensatory. Consequently, different workers may report different values for the yellow and red units for the same oil, and the same workers may report different values for the yellow and red units for the oil examined at different times. To obviate such personal errors, a composite factor is to be used for checking the colour, comprising the sum total of the yellow (Y) units and 5 or 10 times the total of the red (R) units as specified for the oil or fat; (Y + 10 R) is the mode of expressing the colour of moderately dark-coloured oils, such as washed cottonseed oil, whereas (Y + 5 R) is the mode of expressing the colour of other vegetable oils.

14.1.3.2 The dimensions of the cell used and the mode of expressing the colour reading for different oils shall be as given in Table 4.

Table 4 Dimensions of the Cell Used and the Mode of Expressing the Colour Reading for Different Oils

(*Clause* 14.1.3.2)

Oil/Fat	Size-Designation of Cell (in)	Mode of Expressing Colour Reading
Castor	1	Y + 5R
Groundnut	1	Y + 5R
Coconut	1	Y + 5R
Sesame	¼	Y + 5R
Mustard	¼	Y + 5R
Mahua	¼	Y + 5R
Cottonseed (washed and refined)	¼	Y + 10R

14.2 Gardner Colour Scale Method

In this method, the colour of optically clear, yellow/brownish coloured liquid products is measured by means of the Gardner colour scale using colour-measuring instruments. The method uses the Gardner colour scale described in **14.2.2**. The method provides a more precise way of measuring Gardner colour than a visual sample comparison using human eyes. It is applicable to products having colours from Gardner 1 to Gardner 18. The Gardner scale is not applicable to products with colours darker than 18.

14.2.1 *Apparatus and Materials*

14.2.1.1 Colour-measuring instrument, spectrophotometer capable of measuring transmitted colour (0°/180° geometry) and reporting the results in the Gardner colour scale. If such an instrument is not available, one may be used which is capable of measuring transmitted colour and reporting in tristimulus values using standard illuminant C and the 2° observer, described in CIE Publication No. 15 : 2004.

14.2.1.2 Absorption cells, 10 mm light path length recommended, unless a different path length is specified by the instrument manufacturer.

14.2.1.3 Glass tubes, 11 mm path length. Glass test tubes designed for a specific instrument may be used.

14.2.2 *Gardner Colour Standards*

14.2.2.1 *Reagents*

a) Hydrochloric acid, diluted 1 + 17 — Mix 1 volume of concentrated hydrochloric acid, 38 percent (by mass), ρ = 1,19 g/ml, with 17 volumes of water.

b) Potassium hexachloroplatinate solution — Dissolve 790 mg of potassium hexachloroplatinate (K_2PtCl_6) in diluted hydrochloric acid [14.2.2.1 (a)] in a 100 ml volumetric flask. Warm the solution until all the potassium hexachloroplatinate has dissolved. Cool to 20 °C, dilute to the mark with the same hydrochloric acid and mix well. The stock solution prepared in this way shall have tristimulus values which lie within the limits specified in Table 5 when measured in accordance with **14.2.1.1** and **14.2.1.2** (10 mm optical path length) in the spectrophotometer.

Table 5 Tristimulus Tolerance Limits for Potassium Hexachloroplatinate Stock Solution

[*Clause* 14.2.2.1 (b)]

X	*Y*	*Z*
80.9 ± 0.5	87.1 ± 0.5	24.5 ± 1.5

c) *Cobalt (II) chloride solution* — Dissolve 40 g of cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) in 120 g of diluted hydrochloric acid [14.2.2.1 (a)].

d) *Iron (III) chloride solution* — Dissolve 1 000 g of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in 240 g of diluted hydrochloric acid [14.2.2.1 (a)], heating gently if necessary. Adjust the concentration so that the solution has exactly the same colour (visually assessed) as a freshly prepared 30 g/l solution of potassium dichromate (K_2Cr_2O7) in concentrated sulfuric

acid (ρ = 1.84 g/ml). The stock solution prepared in this way shall have tristimulus values X, Y, Z which lie within the limits specified in Table 6 when measured in accordance with to **14.2.1.1** and **14.2.1.2** (10 mm optical path length) in the spectrophotometer.

Table 6 Tristimulus Tolerance Limits for Iron (III) Chloride Stock Solution

[*Clause* 14.2.2.1 (d)]

X	*Y*	*Z*
10.0 ± 2.5	5.3 ± 1.5	0.0 ± 0.2

14.2.2.2 Preparation of liquid colour standards

a) *Gardner colour standards 1 to 8* — Into each of a series of volumetric flasks of the capacities indicated in Table 7, transfer from a microburette the volume of potassium hexachloroplatinate solution [**14.2.2.1 (b)**] shown in the below Table, make each up to the mark with diluted hydrochloric acid [**14.2.2.1 (a)**] and mix well.

Table 7 Composition of Gardner Colour Standards 1 to 8

[*Clause* 14.2.2.2 (a)]

Gardner Colour Standard Number	Volume of Potassium Hexachloroplatinate Solution ml	Volume of Volumetric Flask ml
1	3.48	50
2	5.47	50
3	8.42	50
4	6.58	25
5	9.60	25
6	5.35	10
7	8.10	10
8	10.00	10

b) Gardner colour standards 9 to 18 − Into a series of 100 ml one-mark volumetric flasks, introduce from burettes the volumes of iron (III) chloride solution [**14.2.2.1 (d)**] and cobalt (II) chloride solution [**14.2.2.1 (c)**] shown in Table 8. Make each up to the mark with diluted hydrochloric acid [**14.2.2.1 (a)**] and mix well.

14.2.2.3 *Storage*

Gardner colour standards are stable for 6 months when stored in the dark but should preferably be prepared immediately before use.

14.2.3 *Procedure*

Baseline calibration of the instrument shall be performed following the instrument manufacturer's recommendations. If the material to be tested is cloudy, first filter it (*see* Note). Then, using the same type of glass tube or absorption cell as used for the baseline calibration, fill the glass tube or absorption cell with the product. Take care not to touch the measurement area of the glass tube or absorption cell. If the material shows any visual haziness, remove the haze for example, by filtration, centrifugation, heating, ultrasonic treatment or any other suitable means (*see* Note). If the haziness cannot be removed, the measured value will be unreliable. Then, using the same type of glass tube or absorption cell as used for the baseline calibration, fill the glass tube or absorption cell with the product. Take care not to touch the measurement area of the glass tube or absorption cell. Avoid creating air bubbles when filling the glass tube or absorption cell. If air bubbles are formed and remain trapped, remove them by heating, vacuum, ultrasonic treatment or any other suitable means (*see* Note). Insert the glass tube or absorption cell in the instrument and measure the Gardner colour, following the instrument manufacturer's recommended procedure. Regular checks as per the instrument manufacturer's recommendations should be carried out. These will normally be in the form of checks with certified reference materials.

NOTE — Some sample pre-treatments can change the colour.

Table 8 Composition of Gardner Colour Standards 9 to 18

[*Clause* 14.2.2.2 (b)]

Gardner Colour Standard Number	Volume of Iron (III) Chloride Solution ml	Volume of Cobalt (II) Chloride Solution ml	Volume of Hydrochloric Acid ml
9	3.8	3.0	93.2
10	5.1	3.6	91.3
11	7.5	5.3	87.2
12	10.8	7.6	81.6
13	16.6	10.0	73.4
14	22.2	13.3	64.5
15	29.4	17.6	53.0
16	37.8	22.8	39.4
17	51.3	25.6	23.1
18	100.0	0.0	0.0

14.2.4 *Expression of results*

Report the colour in Gardner colour units to the nearest tenth of a Gardner unit as given by the instrument.

15 DETERMINATION OF IODINE VALUE (WIJS)

15.0 General

The material is treated, in chloroform medium, with a known excess of iodine monochloride solution in glacial acetic acid (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and. the liberated iodine estimated by titration with sodium thiosulphate solution.

15.1 Apparatus

15.1.1 *Iodine Flask*, 250 or 500 ml.

15.1.2 *Burette* 50 ml capacity, graduated in 0.1 ml divisions or automatic.

15.1.3 *Pipette,* 10 ml and 25 ml capacity or automatic.

15.1.4 *Measuring Cylinder,* 50 ml, 100 ml.

15.1.5 *Analytical Balance*, readability 0.0001g weighing precision 0.001g.

15.2 Reagent

15.2.1 *Potassium Dichromate*, conforming to IS 250.

15.2.2 *Concentrated Hydrochloric Acid*, conforming to IS 265.

15.2.3 *Potassium Iodide Solution*, Prepare a fresh solution by dissolving 10 g of potassium iodide free from potassium iodate, in 90 ml of water.

15.2.4 *Starch Solution,* triturate 5 g of starch and 0.01 g of mercuric iodide with 30 ml of cold water and slowly pour it with stirring into one litre of boiling water. Boil for three minutes. Allow to cool and decant off the supernatant clear liquid.

15.2.5 *Standard Sodium Thiosulphate Solution*, approximately 0.1 N. Dissolve approximately 24·8 g of sodium thiosulphate crystals ($Na_2S_2O_3$, $5H_2O$) in water which has been well boiled to free it from carbon dioxide and make up to 1 000 ml, Store the solution in a cool place in a dark. coloured stock bottle with a guard-tube filled with soda lime, after storing the solution for about two weeks, filter, if necessary, and standardize it as prescribed under **15.2.5.1**.

15.2.5.1 Weigh accurately about 5.0 g of finely ground potassium dichromate which has been previously dried to a constant weight at 105±2 °C into a clean one-litre volumetric flask. Dissolve in water, make up to the mark; shake thoroughly and keep the solution in a cool dark place. For standardization of sodium thiosulphate, pipette 25 ml of this solution into a clean glass stoppered 250 ml conical flask or bottle. Add 5 ml of concentrated hydrochloric acid and 15 ml of a 10 percent potassium iodide solution. Allow to stand in the dark for 5 min and titrate the mixture with the solution of sodium thiosulphate, using starch solution as an internal indicator towards the end. The end point is taken when the blue colour changes to green, Calculate the normality (N) of the sodium thiosulphate solution as follows:

$$\frac{25\,W}{49 \cdot 03\,V}$$

where

W = weight in g, of the potassium dichromate; and

V = volume in ml of sodium thiosulphate solution required for the titration.

15.2.6 *Iodine Crystals,* re-sublimed.

15.2.7 *Acetic Acid*, glacial, 99 percent, having a melting point of 14.8 °C and free from reducing impurities. Determine the melting point of the acetic acid and test it for reducing impurities as follows:

a) *Determination of Melting Point* — Take a 15 cm long test-tube and fill it to about two-thirds with the acetic acid. Insert into the acid a thermometer satisfying the requirements specified under 13.1.8 through a cork stopper fitting the test-tube. The amount of acid should be at least double the quantity required to cover the bulb of the thermometer when the bottom of the latter is 12 mm from the bottom of the test-tube. Suspend this tube within a larger test-tube through a cork. Cool the acid by immersing the assembly in ice water until the temperature is 10 °C, then withdraw the assembly from the ice water and stir the acid rather vigorously for a few moments, thus causing the super-cooled liquid to crystallize partially. Take thermometer readings every 15 s and consider as the true melting point, that temperature at which the reading remains constant for at least 2 min.

b) *Test for Reducing Impurities (Potassium Permanganate Test)* — Dilute 2 ml of the acetic acid with 10 ml of water and add 2 drops of 0.1 N potassium permanganate solution and maintain at 27±2 °C. The test shall be taken as having been satisfied if the pink colour is not discharged at the end of two hours.

15.2.8 *Chlorine Gas*, dry.

15.2.9 *Iodine Trichloride* (ICl_3).

15.2.10 *Iodine Monochloride* (ICI), 98 percent chemically pure.

15.2.11 *Wijs Iodine monochloride Solution*, prepare this solution by one of the following three methods, and store *in* a glass-stoppered bottle in a cool place, protected from light:

a) Dissolve 13 g of iodine in one litre of acetic acid, using gentle heat, if necessary, and determine the strength by titration with standard sodium thiosulphate solution. Set aside 50 to 100 ml of the solution and introduce chlorine gas into the remainder until the characteristic colour change occurs and the halogen content is nearly' doubled as ascertained again by titration. If the halogen content has been more than doubled, reduce it by adding the requisite quantity of the iodine-acetic acid solution. A light excess of iodine does no harm, but avoid an excess of chlorine.

   Example

   If the titration of 20 ml of original iodine-acetic acid solution requires 22 ml of standard sodium thiosulphate, 20 ml of the finished Wijs solution should require between 43 and 44 ml (and not more than 44 ml) of the same sodium thiosulphate solution.

b) Dissolve 8 g of iodine trichloride in approximately 450 ml of acetic acid. Dissolve separately 9 g of iodine in 450 ml of acetic acid, using heat, if necessary. Add gradually the iodine solution to the iodine trichloride until the colour has changed to reddish brown. Add 50 ml more of iodine solution and dilute the mixture with acetic acid till 10 ml of the mixture is equivalent to 20 ml of standard thiosulphate solution when the halogen content is estimated by titration in the presence of an excess of potassium iodide and water. Heat the solution to 100 ºC for 20 minutes, and cool. Prevent access of water vapour in preparing the solution.

c) Dissolve 10 ml of iodine monochloride in about 1 800 ml of glacial acetic acid and shake vigorously. Pipette 5 ml of this, add 10 ml of potassium iodide solution and titrate with 0.1 N standard sodium thiosulphate solution, using starch solution as indicator. Adjust the volume of the solution till it is approximately 0.2 N.

15.2.12 *Chloroform* — Inert to Wijs solution.

15.3 Procedure

Melt the sample if it is not already completely liquid, and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample as well as the glass apparatus used is absolutely clean and dry. Weigh accurately, by difference, an appropriate quantity of the oil or fat between the limits indicated in col 2 and 3 of below Table 9, into a clean dry 500 ml iodine flask or well ground glass-stoppered bottle to which 25 ml of-choloroform have been added, and agitate to dissolve the contents. The weight of the sample shall be such that there is an excess of 50 to 60 percent of Wijs solution over that actually needed. Add 25 ml of the Wijs solution and replace the glass stopper after wetting with potassium iodide solution; swirl for intimate mixing, and allow to stand in the dark for 30 minutes in the case of non-drying and semi-drying oils and one hour in the case of drying oils. Carry out a blank test simultaneously under similar experimental conditions. After standing, add 15 ml of potassium iodide solution and 100 ml of water, rinsing in the stopper also, and titrate the liberated iodine with standard sodium thiosulphate solution, swirling the contents of the bottle continuously to avoid any local excess until the colour of the solution is straw yellow. Add one millilitre of the starch solution and continue the titration until the blue colour formed disappears after thorough shaking with the stopper on.

Table 9 Weight of Oil or Fat for Determination of Iodine Value

(*Clause* 15.3)

Iodine Value Expected	Weight in g of Sample		Weighing Accuracy
	Maximum	Minimum	
(1)	(2)	(3)	(4)
Less than 3	10	10	±0.001
5	6.3460	5.0770	±0.0005
10	3.1730	2.5384	±0.0002
50	0.6612	0.5288	±0.0002
100	0.3173	0.2538	±0.0001
150	0.2125	0.1700	±0.0001
200	0.1586	0.1269	±0.0001

15.4 Calculation

$$\text{Iodine value} = \frac{12.69\,(B - S)\,N}{W}$$

where

B = Volume in ml, of standard sodium thiosulphate solution required for the blank;

S = volume in ml, of standard sodium thiosulphate solution required for the sample;

N = normality of the standard sodium thiosulphate solution; and

W = weight in g, of the material taken for the test.

16 DETERMINATION OF SAPONIFICATION VALUE

16.0 General

The material is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali consumed for saponification is determined by titrating the excess alkali with standard hydrochloric acid.

16.1 Apparatus

16.1.1 *Conical Flasks*, 250 ml capacity having a ground neck.

16.1.2 Reflux *Condenser*, any efficient reflux condenser, at least 65 cm long with a glass joint fits the conical flask (**16.1.1**).

16.1.3 *Water-Bath or Electric Hot-Plate, with Rheostat Control*

16.1.4 *Burette 50 ml capacity, graduated in 0.1 ml divisions or automatic*

16.1.5 *Pipette 25 ml capacity or automatic*

16.1.6 *Analytical balance, readability 0.0001 g weighing precision 0.001 g*

16.2 Reagent

16.2.1 *Alcoholic Potassium Hydroxide Solution*, Dissolve 35 to 40 g of potassium hydroxide in 20 ml of distilled water, and add sufficient aldehyde-free rectified spirit (conforming to IS 323) to make up to 1 000 ml. Allow to stand overnight, decant the clear liquid and keep in a bottle closed tight with a cork or rubber stopper.

16.2.2 *Aldehyde-Free Rectified Spirit*, may be prepared according to either, of the two methods given in **15.2.2.1** and **15.1.2.2**.

16.2.2.1 *Method I*, re-distil rectified spirit (conforming to IS 323) over solid caustic soda or caustic potash, add 2 to 3 g of meta phenylenediamine hydrochloride per litre of rectified spirit, digest at ordinary temperature for several days or under a reflux condenser on a steam-bath for several hours and distil slowly) rejecting the first 100 ml and the last 200 ml of the distillate.

16.2.2.2 *Method II,* reflux 1-2 litres of rectified spirit (conforming to IS 323) for 30 min in a round-bottom flask with 10 g of caustic potash and 6 g of granulated aluminium (or aluminium foil). Distil and collect one litre after discarding the first 50 ml.

16.2.3 *Phenolphthalein Indicator Solution*, Dissolve 1.0 g of phenolphthalein in 100 ml of rectified spirit (conforming to IS 323).

NOTE — When testing oils or fats which give dark-coloured soap solutions, the observation of the end point of the titration may be facilitated either:

a) by using thymolphthalein, or alkali blue 6 B in place of phenolphthalein, or
b) by adding one millilitre bf a 0.1 percent (*w*/*v*) solution of methylene blue in water to each 100 m1 of phenolphthalein indicator solution before the titration.

16.2.4 *Standard Hydrochloric Acid,* approximately 0·5 N.

16.3 Procedure

Melt the sample, if it is not already liquid, and filter through a filter paper to remove any impurities and the lent traces of moisture. Make sure that the sample is completely dry, Mix the sample thoroughly, and weigh accurately by difference about l.5 to 2·0 g of the sample in a conical flask. Add 25 ml of the alcoholic potassium hydroxide solution and connect the reflux air condenser to the flask. Heat the flask on a water-bath or an electric hot-plate for not more than one hour, Boil gently but steadily until the sample is completely saponified as indicated by absence of any oily matter and appearance of clear solution. After the flask and condenser have cooled somewhat, wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein.

Add about one millilitre of phenolphthalein indicator solution, and titrate with standard hydrochloric acid until the pink colour just disappears. Prepare and conduct a blank determination at the same time.

16.4 Calculation

$$\text{Saponification value} = \frac{56.1\,(B-S)\,N}{W}$$

where

B = volume in ml, of standard hydrochloric acid required for the blank;

S = volume in ml, of standard hydrochloric acid required for the sample;

N = normality of the standard hydrochloric acid; and

W = weight in g, of the material taken for the test.

17 DETERMINATION OF ACETYL VALUE AND HYDROXYL VALUE

17.0 General

Two methods have been prescribed, namely, Method A and Method B. Being simpler, Method B is preferred.

17.0.1 *Method A*

A sample of oil or at is acetylated by refluxing with acetic anhydride and the excess anhydride is decomposed with water and sodium bicarbonate solution. The saponification value of the washed and dried acetylated oil is determined as in **16**.

17.0.2 *Method B*

The process consists in acetylating the oil or fat with a measured quantity of acetic anhydride in pyridine decomposing the excess anhydride by boiling with water and then, after the addition of sufficient butyl

alcohol to give a homogeneous solution, titrating with alcoholic alkali. A control test with the acetic anhydride and pyridine without the oil or fat provides a measure of the acetic anhydride available for acetylation; a similar test with the oil or fat and the pyridine without the acetic anhydride provides a measure of the free fatty acid present. From the figures obtained" the acetyl value or the hydroxyl value of the fat is calculated.

17.1 Method A

17.1.1 *Apparatus*

17.1.1.1 *Beaker,* 800 ml capacity.

17.1.1.2 *Separating funnel,* 500 ml capacity.

17.1.1.3 *Conical flasks*, 250 to 300 ml capacity.

17.1.1.4 *Reflux condemn* — any efficient reflux condenser, at least 65 cm long.

17.1.1.5 *Water bath or electric hot-plate with rheostat control or heating mantle*

17.1.2 *Reagent*

17.1.2.1 *Acetic anhydride* — Containing 95 to 100 percent (by weight) or the actual acetic anhydride. Determine the acetic anhydride content as follows:

a) Weigh accurately about 2 g of the acetic anhydride into a 200 ml glass-stoppered conical flask, cool in ice and add 5 ml of freshly distilled aniline. Insert the glass stopper immediately, shake vigorously and allow to stand at room temperature for 30 min. Wash down the sides of the flask with 50 ml of ice-cold water. Mix well and titrate with previously standardized 1 N sodium hydroxide solution, using phenolphthalein as indicator, until the pink colour persists for 10 min.

$$A = \frac{\text{volume in ml of 1 N sodium hydroxide solution required}}{\text{Weight in g of acetic anhydride taken for the test}}$$

b) Weigh accurately about 2 g of the acetic anhydride into another flask, add 50 ml of water, allow to stand for 30 min and titrate with the standard sodium hydroxide solution to the same end point as above using phenolphthalein as indicator.

$$B = \frac{\text{Volume in ml of 1 N sodium hydroxide solution required}}{\text{Weight in g of acetic anhydride taken for the test}}$$

Acetic anhydride, percent by weight = 10·209 *(B – A)*

17.1.2.2 *Sodium bicarbonate solution*, freshly prepared 0.5 percent (*w/v*) and neutral to litmus.

17.1.2.3 *Anhydrous sodium sulphate*

17.1.2.4 *Alcohol potassium hydroxide solution*, 0·5 N.

17.1.2.5 *Phenolphthalein indicator solution*, Dissolve 0.1 g in 100 ml of 60 percent rectified spirit.

17.1.2.6 *Standard hydrochloric acid*, approximately 0.5 N.

17.1.3 *Procedure*

Weigh accurately about 10 g of the material in a conical flask, add 20 ml of acetic anhydride and boil the mixture under a reflux air condenser for about 2 h. Pour the mixture into a beaker containing 500 ml of water and boil for 15 min. Bubble a stream of carbon dioxide or nitrogen through the mixture during boiling to prevent bumping. Discontinue boiling, cool slightly, and remove the water with a siphon. Add another 500 ml of water and boil again. Discontinue boiling, cool and transfer the contents of the beaker to a separating funnel and reject the lower layer. Wash the acetylated sample successively (a) three times with 50 ml of water, (b) twice with 50 ml of sodium bicarbonate solution, and (c) twice with 50 ml of warm water (60 to 70 °C). Drain and remove as much of the water as possible» and then transfer the acetylated sample to a beaker and add approximately 5 g of anhydrous sodium sulphate. Allow to stand for about one hour with occasional stirring, Filter through a dry filter paper, preferably in an oven at 100 to 110 °C, remove the filter paper and keep the sample in the oven until it is thoroughly dry. Determine the saponification values of the original material and the acetylated product by the procedure described under **16.3**.

17.1.4 *Calculation*

a) Acetyl value = $\frac{S' - S}{1.000 - 0.000\,75\,S}$

b) Hydroxyl value = $\frac{S' - S}{1.000 - 0.000\,75\,S'}$

where

S' = saponification value after acetylation, and

S = saponification value before acetylation (*see* 16).

17.2 Method B

17.2.1 *Reagents*

17.2.1.1 *Pyridine* — Reflux with powdered barium oxide and distil. Use the fraction distilling above 114 °C.

17.2.1.2 *Acetic anhydride*

17.2.1.3 *Acetylating agent* — Mix one volume of acetic anhydride and seven volumes of pyridine.

17.2.1.4 *Alcoholic sodium hydroxide, solution* — Prepare by dissolving sufficient aqueous caustic soda (60 percent *w/v*) in 95 percent alcohol to make a 0.30 to 0.35 N solution. Remove the precipitated carbonate by filtering.

The solution should be standardized against standard acid in the presence of phenolphthalein before use. The solution remains colourless for a long time if kept below 25 °C.

17.2.1.5 *Normal butyl alcohol*

17.2.1.6 *Phenolphthalein solution* — Dissolve 0.1 g in 100 ml of 60 percent rectified spirit.

NOTE — In the determination upon substances giving dark-coloured soap solution, observation of the end point of the titration may be facilitated either (a) by the substitution of thymolphthalein or alkali blue 6B for phenolphthalein or (b) by the addition of one millilitre of 0.1 percent solution of methylene blue to each 100 ml of the phenolphthalein solution before the titration.

17.2.2 *Apparatus* — A round-bottomed acetylation flask made of resistance glass of capacity 150 to 200 ml with a 100 cm ground-in air condenser tube.

17.2.3 *Procedure*

17.2.3.1 Weigh accurately 0.5 to 3.0 g of fat in the acetylation flask (the following weights serve as a rough guide for different types of materials: fatty alcohols 0.5 to 0.7 g, castor oil about one gram and ordinary oils 2 to 3 g). Measure, or preferably weigh, from a 10 ml burette into the flask, 5 ml of the pyridine-acetic anhydride mixture. Add the mixture dropwise, and allow no time for drainage. Before attaching the condenser, moisten the neck of the flask with pyridine to act as a seal, and make sure that the seal is maintained during the acetylation.

17.2.3.2 Mix the fat and the acetylating agent by shaking well, add one or two small pieces of pumice, and boil the contents of the flask 1 gently during 60 min, maintaining the boiling so that the vapour rises no higher than the bottom end of the condenser. In this way, the pyridine seal needs renewal less often, and there is less tendency for the ground-in joint to seize.

17.2.3.3 Cool the flask to about 50 °C and with a rotary motion to assist in washing the condenser tube, add 5 ml of distilled water, from the, top of the condenser. Shake the mixture well, and then boil it gently for 5 to 10 minutes, shaking the flask two or three times during the boiling.

17.2.3.4 After cooling the flask and the contents to room temperature and before detaching the condenser, wash the condenser with 30 ml of butyl alcohol. Detach the condenser and wash the neck and mouth of the flask and the tip of the condenser with a further 20 ml and then, if the contents of the flask are not homogeneous, add butyl alcohol until they become homogeneous. Titrate the free acetic acid with carbonate-free 0.35 N sodium hydroxide solution, in the presence of a few drops of phenolphthalein as indicator.

17.2.3.5 Carry out the same series of operations with 5 ml of pyridine acetic anhydride mixture alone, also with a corresponding weight of the oil or fat plus 5 ml of pyridine.

NOTE — Owing to the presence of vapour, of pyridine and butyl alcohol, it is preferable to carry out the tests in a fuming chamber.

17.2 Calculation

a) Hydroxyl value = $\dfrac{56.1\ NY}{W}$

b) Acetyl value, $A = \dfrac{\mathrm{H}}{1+0.000\,75\ H}$

where

N = normality of sodium hydroxide solution; and

Y = volume of sodium hydroxide solution in ml corresponding to the amount of acetylated fat formed = $a + b - c$.

where

a, b and c are respectively the volumes in ml of sodium hydroxide required by blank with pyridine-acetic anhydride mixture, fat plus pyridine, and fat plus pyridine plus acetic anhydride; and

W = weight in g of fat or oil taken for test.

NOTE — It is to be noted that a slight increase in the acetyl value has been found to occur with increasing free fatty acid content of the sample.

18 DETERMINATION OF ALLYL ISO THIOCYANATE

18.0 General

The oil obtained from black mustard seeds contains sinigrin and myrosin which, after maceration with water, yields a volatile oil, the major constituent of which is allyl isothiocyanate. The oil obtained from white mustard seeds contains acrinyl isothiocyanate which is much less volatile than allyl isothiocyanate.

18.0.1 *Method*

The allyl isothiocyanate in the oil is steam distilled into a known excess of silver nitrate solution, and the excess of silver nitrate solution is determined by titration with standard ammonium thiocyanate solution.

18.1 Apparatus

18.1.1 *Distillation Flask* — 500 ml round-bottomed flask.

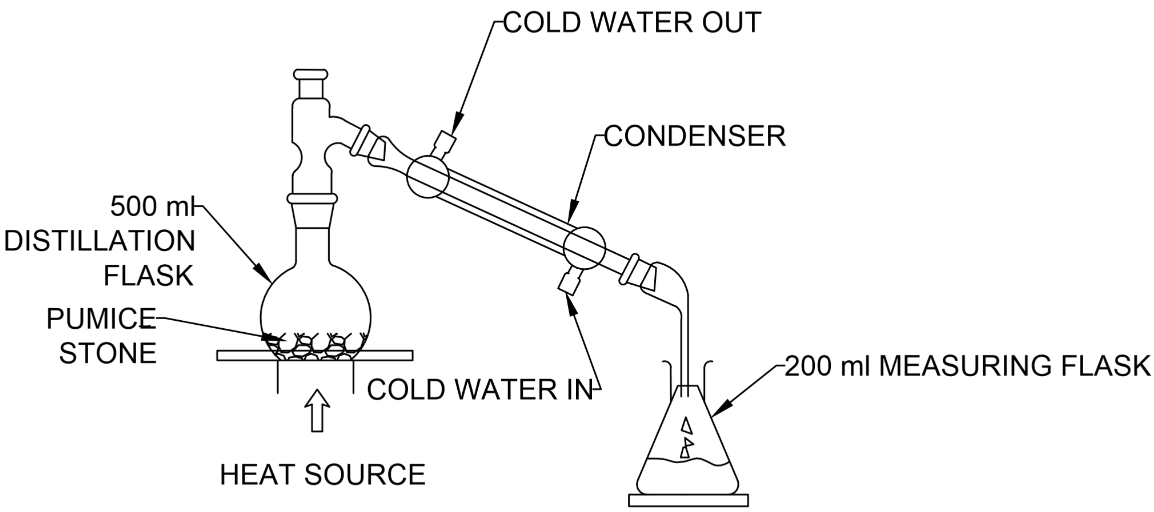


FIG. 7 DISTILLATION APPARATUS FOR ALLYL THIOCYANATE

18.1.2 *Any efficient Reflux Condenser* — Approximately 90 cm long.

18.1.3 *Measuring Flask* — 200 ml capacity.

18.1.4 *Water-Bath*

18.1.5 *Steam generator*

18.1.6 *Burette 50 ml capacity, graduated in 0.1 ml divisions or automatic*

18.1.7 *Pipette 25 ml capacity or automatic*

18.1.8 *Analytical balance, readability 0.0001 g weighing precision 0.001 g*

18.2 Reagent

18.2.1 *Ethyl Alcohol,* 95 percent (by volume) or rectified spirit (conforming to IS 323) neutral to phenolphthalein.

18.2.2 *Silver Nitrate Solution*, approximately 0.1 N.

18.2.3 *Ammonium Hydroxide Solution*, 10 percent (*w/v*).

18.2.4 *Nitric Acid*, conforming to IS 264.

18.2.5 *Ferric Ammonium Sulphate Indicator*, 0·1 percent solution in water.

18.2.6 *Standard Ammonium Thiocyanate Solution*, approximately 0.1 N.

18.3 Procedure

Weigh accurately about 5 g of the material into a 500 ml distillation flask and add to it 25 ml of ethyl alcohol, 250 ml of water and a few pieces of pumice stone. Distil the mixture in steam and collect the distillate in a 200 ml measuring flask containing exactly 25 ml of silver nitrate solution and 10 ml of ammonium hydroxide solution. Collect as rapidly as possible about 150 ml of the distillate. Attach the reflux air condenser to the measuring flask and heat the mixture-for about one hour on a boiling water-bath. Cool to room temperature. Add water to make up to 200 ml and filter the contents after shaking with Whatman no. 1 filter paper. Take 100 ml of the filtrate, add 6 ml of nitric acid and a few drops of ferric ammonium sulphate indicator, and titrate with standard ammonium thiocyanate solution until a permanent red colour is obtained. Carry out a blank test at the same time.

18.4 Calculation

Allyl isothiocyanate, percent by weight

$$= \frac{9.915\,(B-S)\,N}{W}$$

where

B = volume in ml, of standard ammonium thiocyanate solution required for the blank determination;

S = volume in ml, of standard ammonium thiocyanate solution required for the sample;

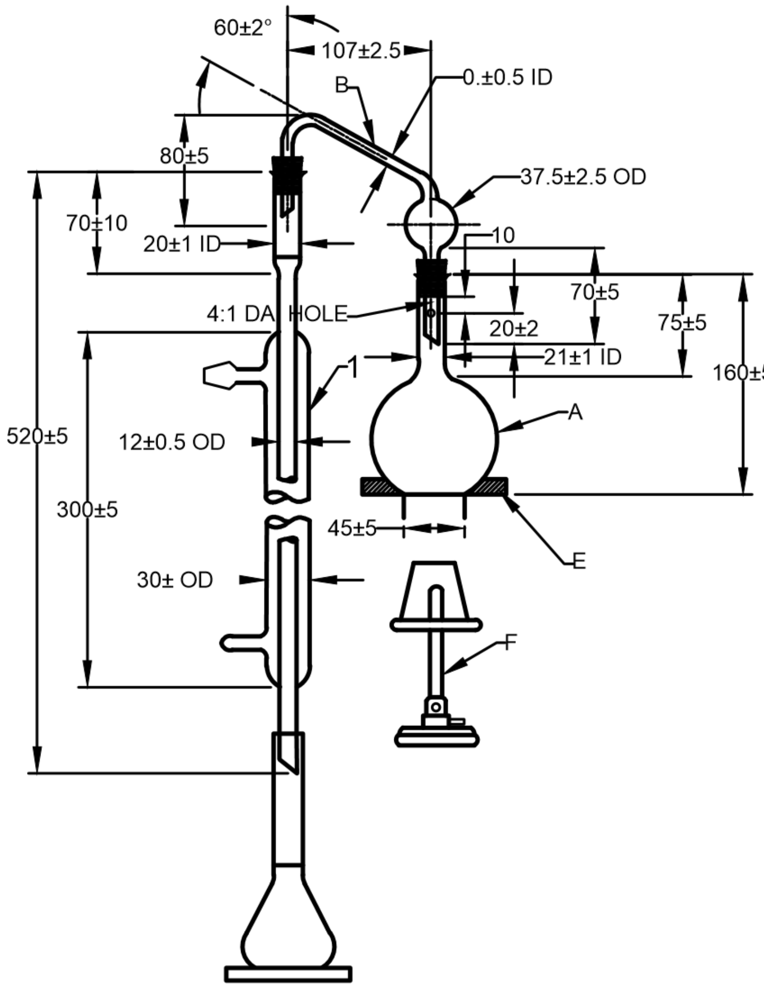
N = normality of standard ammonium thiocyanate solution; and

W = weight in g, of the sample taken for the test.

19 DETERMINATION OF REICHERT-MEISSL VALUE

19.0 General

The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulphuric acid. The volatile acids are



All dimensions are in millimetres

FIG. 8 REICHERT-MEISSL DISTILLATION APPARATUS

immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

19.1 Apparatus

The assembly of the apparatus for distillation is shown in Fig. 8, and details of the constituent parts are given below:

a) *Flat Bottom Boiling Flask* — The flask *(A)* shall be made of resistance glass and shall conform to the dimensions given in Fig. 8.
b) *Still-Head* — The still-head *(B)* shall be made of glass tubing of wall thickness 1.25 ± 0.25 mm and shall conform to the shape and dimensions shown in Fig. 8.

   A rubber stopper, fitted below the bulb of the longer arm of the still-head, and used for connecting it to the flask, shall have its lower surface 10 mm above the centre of the side-hole of the still-head.
c) *Condenser* — The condenser (*C*) shall be made of glass and shall conform to the dimensions indicated in Fig 8.
d) *Receiver* — The receiver (*D*) shall be a flask, with two graduation marks on the neck, one at 100 ml and the other at 110 ml.
e) *Asbestos Board* — An asbestos board (*E*), 120 mm diameter, 6 mm in thickness, with a circular hole about 65 mm in diameter, shall be used to support the flask over the burner. During distillation, the flask shall fit snugly into the hole in the board to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos beard may conveniently be prepared by beveling the edge of the hole, soaking in water, moulding the edge with a flask, and drying.
f) *Bunsen Burner* — The burner *(F)* should be sufficiently large to allow the distillation to be completed in the time specified in **19.3.2**.

19.2 Reagents

19.2.1 *Glycerine*, conforming to AR Grade of IS 1796.

19.2.2 *Concentrated Sodium Hydroxide Solution*, 50 percent *(wlw)*. Dissolve sodium hydroxide in an equal weight of water and store the solution in a bottle protected from carbon dioxide. Use the clear portion free from deposit.

19.2.3 *Pumice Stone Grains*, approximately 1·4 to 2.0 mm in diameter.

19.2.4 *Dilute Sulphuric Acid Solution*, approximately 1·0 N.

19.2.5 *Sodium Hydroxide Solution*, 0.1 N solution in water, accurately standardized.

19.2.6 *Phenolphthalein Indicator*, Dissolve 0.1 g of phenolphthalein in 100 ml of 60 percent rectified spirit.

19.3 Procedure

19.3.1 Weigh accurately 5·00 ± 0·01 g of the filtered oil or fat into the boiling flask. Add 20 g of glycerin and 2 ml of the concentrated sodium hydroxide solution from a burette to which access of carbon dioxide is prevented and whose orifice is wetted before running in the liquid, the first few drops from the burette being rejected. Heat the flask and its contents with continuous shaking on a gauze over the naked flame until the fat, including any drops adhering to the upper parts of the flask, has been saponified and the liquid becomes perfectly clear. Avoid overheating during this saponification. Cover the flask with a watch-glass, and allow the flask to cool a little. Add 90 ml of boiling distilled water which has been vigorously boiled for about 15 min. After thorough mixing, the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating overheating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may sometimes be dark and not clear.

19.3.2 Add 0·6 to 0·7 g of pumice stone grains and 50 ml of dilute sulphuric acid, and immediately connect the flask with the distilling apparatus. Place the flask on the asbestos board so that it fits snugly into the aperture. This will prevent the flame from impinging on the surface of the flask above the level of the liquid and avoid superheating of the system. After the fatty acids have melted and separated into a clear liquid layer on gentle warming, heat the flask without altering the flame so that 110 ml of liquid distils over in the course of 19 to 21 min. The distillation is considered to begin when the first drop forms in the still-head. Keep the water flowing in the condenser at a sufficient speed to maintain the temperature of the outgoing water from the condenser between 15 °C and 20 °C. Collect the distillate in a graduated flask.

19.3.3 As soon as 110 ml have distilled over, stop heating the boiling flask and replace the graduated flask by a measuring cylinder of about 25 ml capacity to catch drainings (for Polenske value). Close the 110 ml graduated flask with the stopper, and, without making up the contents, place it in a water-bath at 15 °C for 10 minutes, making sure that the 100 ml graduation mark is below the level of the water. Swirl round the contents of the flask from time to time. Dry the outside of the flask and then mix the distillate by closing the flask and inverting it four or five times, but do not shake. Filter through a dry 9 cm Whatman No. 4 filter paper. Reject the first 2 or 3 ml of the filtrate and collect the rest in a dry flask.

NOTE — The filtrate should be free from insoluble fatty acids. When operating on coconut oil, it has been noticed that this is not easily achieved. In cases where liquid insoluble fatty acids pass through the filter, transfer the filtrate to a separating funnel and after the separation of the lower aqueous layer, add the insoluble acids to the mail bulk of insoluble acids.

19.3.4 Pipette 100 ml of the filtrate in a titration flask, add 0.1 ml of phenolphthalein indicator solution and titrate with standard (0.1 N) sodium hydroxide solution until the liquid becomes slightly pink.

19.3.5 Run a blank test without the fat but using the same quantities of reagents and following the same procedure. During the heating with caustic soda, avoid overheating which will be indicated by the darkening of the solution.

19.4 Calculation

$$\text{Reichert-Meissl value} = (A - B) \times N \times 11$$

where

A = volume in ml, of standard sodium hydroxide solution required for the test;

B = volume in ml, of standard sodium hydroxide solution required for the blank; and

N = normality of standard sodium hydroxide solution.

NOTE — At high altitudes, that is, low barometric pressures, the Reichert-Meissl value determination, are lower than those determined at atmospheric pressure.

Since the Reichert-Meissl value varies with atmospheric pressure, correction, for the value arc necessary when the determinations are made at places above lea-level or when barometric pressure it fluctuating widely from 760 mm. The corrected Reichert-Meissl value shall be calculated follows;

$$\text{Corrected Reichert Meissl value} = \frac{\left(RM - 10\right) \log 160}{log\, \text{P} + 10}$$

where

RM = observed Reichert-Meissl value; and

P = barometric pressure in rom of mercury at the place and time of determination.

20 DETERMINATION OF POLENSKE VALUE

20.0 General

The condenser, the 25 ml cylinder and the receiver used in the Reichert-Meissl value determination are washed into the filter paper through which the distillate was filtered for that determination. After rinsing, the residue on the filter paper is taken up with ethyl alcohol and titrated with standard sodium hydroxide solution.

20.1 Reagents

All the reagents required for determining the Reichert-Meissl value (*see* **19.1**) as well as the following are required.

20.1.1 *Ethyl-Alcohol,* 90 percent by volume, and neutral to phenolphthalein.

20.2 Apparatus

The same apparatus as that required for the determination of Reichert-Meissl value (*see* **19.2**).

20.3 Procedure

After determining the Reichert-Meissl value, detach the still-head and wash the condenser with three successive l.5 ml portions of cold distilled water, passing each washing separately through the measuring cylinder, the 110 ml flask with stopper, and the filter paper nearly filling the paper each time to the brim and draining each washing before filtering the next. The last 10 ml of wash-water should require not more than one drop of 0.1 N sodium hydroxide solution for neutralization. Discard all the washings.

Dissolve the insoluble acids by three similar washings of the condenser, the measuring cylinder, the 110 ml flask with stopper, and the filter paper with 15-ml portions of ethyl alcohol. Combine the Alcoholic washings in a clean flask (the total volume thus amounting to 45 ml), add 0.25 ml of phenolphthalein indicator solution, and titrate with standard (0.1 N) sodium hydroxide solution until the solution turns slightly pink.

20.4 Calculation

$$\text{Polenske value} = 10\ VN$$

where

V = volume in ml, of standard sodium hydroxide solution required for the test; and

N = normality of standard sodium hydroxide solution.

NOTE — Like Reichert-Meisal value, the Polenske value also varies when determination are done at atmospheric pressure differing widely from 760 mm (*see* **19.4**). The corrected Polenske value shall be calculated as follow:

$$\text{Corrected Polenske value} = \frac{PV\ (760 - 45)}{\text{P} - 45}$$

where

PV = observed Polenske value; and

P = barometric pressure in mm of mercury at die place and time or determination.

21 DETERMINATION OF PEROXIDE VALUE

21.0 General

The peroxide value is a measure of the peroxides contained in a sample of fat, expressed as milli equivalents of peroxide per 1 000 grams of the material.

21.1 Outline of the Method

The material in an acetic acid-chloroform medium, is treated with an aqueous solution of potassium iodide. The liberated iodine is titrated with standard sodium thiosulphate solution.

21.2 Apparatus

21.2.1 *Pipette* — Graduated, 1 ml capacity.

21.1.2 *Conical Flask* — Glass-stoppered, 250 ml capacity.

21.3 Reagents

21.3.1 *Acetic Acid-Chloroform Solution or Acetic acid-iso-octane Solution* — Mix three parts by volume of glacial acetic acid, with 2 parts by volume of chloroform/*iso-octane*.

21.3.2 *Potassium Iodide Solution* — Saturated. Prepare saturated solution of potassium iodide in recently boiled distilled water. Store in the dark.

21.3.3 *Sodium Thiosulphate Solution* 0.1 N. accurately standardized.

21.3.4 *Sodium Thiosulphate Solution* — 0.01 N. This solution is prepared by diluting 100 ml of accurately standardized solution of 0.1 N sodium thiosulphate to 1000 ml with freshly boiled and cooled distilled water.

21.3.5 *Starch Solution* — 1 percent.

21.4 Procedure

21.4.1 Weigh 5.00 ± 0.05 g of sample of fat in a 250 ml glass stoppered conical flask and then add 30 ml of the acetic acid-chloroform or acetic acid-isooctane solution. Swirl the flask until the sample is dissolved. Add 0·5 ml of saturated potassium iodide solution. Allow the solution to stand exactly one minute with occasional shaking and then add 30 ml or distilled water. Titrate with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. Continue titration until the yellow colour almost disappear Add 0.5 ml of starch solution and continue titration till the blue colour just disappear.

21.4.2 If the titre value is less than 0.5 ml, repeat the determination using 0.01 N sodium thiosulphate solution.

21.4.3 Conduct a blank determination of the reagents in the same way. The titration in blank determination should not exceed 0·1 m1 of the 0.1 N sodium thiosulphate solution.

21.5 Calculation

Peroxide value as milli-equivalents per 1 000 grams

$$\text{Sample} = \frac{(S - B) \times N \times 1000}{g}$$

where

S = volume in ml, of sodium thiosulphate solution used up by the sample;

B = volume in ml, of the sodium thiosulphate solution used up in the blank determination;

N = normality of the sodium thiosulphate solution; and

g = weight in g, of the sample.

22 TEST FOR THE PRESENCE OF OIL SOLUBLE COLOURS IN OILS AND FATS

22.0 General

Oil soluble colours are colours (both natural including nature identical and synthetic) soluble in oils and fats.

22.1 Outline of the Method

Oils, fats and other interfering substances are removed from the colours by solvent partition technique using dimethylformamide and hexane (3 : 1), followed by alumina adsorption. The colours are detected by reversed phase paper chromatography.

22.2 Apparatus

22.2.1 *For Extraction*, a mechanical shaker.

22.2.2 *For Column Chromatography*, a chromatographic tube made of glass (2·5 cm in diameter and 30 cm in length).

22.2.3 *Separating Funnel*, 250 ml capacity.

22.3 Reagents

22.3.1 *Hexane*

22.3.2 *Dimethyl formamide*

22.3.3 *Sodium Chloride Solution — Saturated*

22.3.4 *Alumina Neutral, Brockmann, Activity 1, for example, made by National Chemical Laboratory, Pune or of Equivalent Quality* — Activated at 100 °C for 4 hours.

22.3.5 *Diethyl Ether*

22.3.6 *Ethyl Alcohol*

22.3.7 *Ammonium Hydroxide Solution*

22.3.8 *Methanol*

22.3.9 *Petroleum Ether* — B. P. 40 to 60 °C.

22.3.10 *Liquid Paraffin*

22.3.11 *Whatman No. 1 Filter Paper (Reversed Phase)* — Prepared by soaking the paper in 5 percent liquid paraffin in petroleum ether and subsequent drying in air.

22.3.12 *Oil Soluble Colours* — Butter yellow, Cres orange GN, Sudan IV, Gress Yellow GRN type, etc.

22.3.13 *Sodium Hydroxide Solution* — 5 percent (*m/v*).

22.3.14 *Sulphuric Acid Solution* —13 N.

22.3.15 *Stannous Chloride Solution in Hydrochloric Acid* — Dissolve 112·8 g of stannous chloride in 170 ml of concentrated hydrochloric acid using heat if necessary. Dilute with water to 1 litre and add a few pieces of tin metal.

22.3.16 *Boric Acid Solution* — Dissolve 4 g of boric acid in 100 ml of concentrated hydrochloric acid (relative density 1.19).

22.3.17 *Anhydrous Sodium Sulphate*

22.3.18 *Saturated Solution of Antimony Trichloride in Chloroform*

22.3.19 *Solvent Mixture* — Diethyl Ether: Alcohol: water : 5 : 3 : 2.

22.3.20 *Solvent Mixture* — 80 percent ethyl alcohol in water.

22.4 Procedure

22.4.1 *Extraction*

Take about 2 to 3 g of the sample in a conical flask, add approximately 50 ml of hexane and then shake for about 10 min in a mechanical shaker. Filter it and concentrate the filtrate to about 10 ml.

22.4.1.1 *Qualitative test for presence of synthetic colours*

Divide a portion of the hexane layer into 4 parts and treat with either 13 N sulphuric acid or concentrated hydrochloric acid and water mixtures 4 : 1, 2 : 1 or 1 : 1 respectively. If the acid layer or the whole extract changes shade or more particularly develops pink to reddish violet colour, the synthetic oil soluble colour may be suspected.

22.4.2 *Separation of Colours from Interfering Materials*

Transfer the concentrated extract to a separating funnel and add to it dimethyl formamide (DMF) about three times the volume of the concentrated extract and shake vigorously. Keep it for a while for separation of the layers. The DMF layer contains the natural colour as well as the synthetic colours (if present), leaving oils and fats in the hexane layer. Now take the DMF layer and again shake it with hexane after addition of about 20 ml of water. Retransfer colours (both natural and synthetic) this time to the hexane layer. If emulsion is formed add saturated sodium chloride solution. Concentrate the hexane layer and preferably repeat the above mentioned procedure. If oils and fats still linger in the sample, these will float above the coloured extract when the hexane layer is concentrated to 0.5 ml. If oil is present repeat the above procedure till free from oil. Make the hexane solution to 10 ml, if curcumin or turmeric is suspected, shake the residual DMF layer after extraction with and separation of hexane with diethyl ether and an extra amount of water, if necessary and collect the ether layer. Add the ether layer to the previous hexane. By this treatment any minute curcumin and turmeric will come to the ether layer.

22.4.3 *Separation of Natural Colours from Synthetic Oil Soluble Colours*

Clamp the chromatographic tube and pour 40 ml of hexane with the no greasy stopcock in closed position. Place a cotton plug (if no fritted glass is fixed in the tube) firmly at the bottom of the tube. Pour 40 ml of hexane followed by anhydrous sodium sulphate to form a 2 to 3 cm layer. Now open the stopcock to give a trickle of solvent and tap the tube. Pour alumina sufficient to give a column of 10 cm length into the solvent with constant tapping. This will give a channel-free column. It is important that the top of the alumina is always under the solvent. When the solvent layer reaches about 1 cm above the alumina, pour the hexane extract of colours-carefully into the tube. Collect the eluent in a beaker. Continue the elution with four batches of 10 ml hexane. Concentrate the eluate containing oil soluble synthetic colours. After elution with hexane pass 40 ml of another eluting solvent, namely, petroleum ether: acetone (1 : 1) for complete elution.

22.4.4 *Detection of Synthetic Oil Soluble Colours*

Cut a reversed phase chromatography paper to a size of 20 × 20 cm, then draw a straight line by a lead pencil on the paper leaving away 3 cm from the bottom. Spot the concentrated extract (say 10 µl more according to the colour present on the solution) and also some known oil soluble colours on the line drawn on the reversed phase chromatography paper leaving 1 cm distance from each other. Then make the paper to a cylindrical shape, staple it and dip in anyone of the solvents (*see* **22.3.19** and **22.3.20**) for ascending chromatography. Run for 10 cm, the development in the solvent given under **22.3.19** takes 45 min and that in solvent prescribed under **22.3.20** takes one hour. Take out the paper, dry in air and detect the colour by comparing with the known colour in respect of shade and R_f values.

22.4.5 *Detection of Natural Colours*

Elute the natural colours absorbed on the column by the solvent methanol : ammonium hydroxide (9 : 1) and then examine for natural colours. If colour of the column changes to red. Violet after passing the above mentioned solvent, it confirms the presence of curcumin or turmeric.

22.4.5.1 *Detection of annatto, turmeric and curcumin*

Evaporate the extracted layer (*see* **22.4.5**) to dryness, add 10 ml petroleum ether and shake a 7 ml portion of the solution with sodium hydroxide solution. Take out the alkali layer and divide it into two portions to check for the presence of annatto, turmeric and curcumin. Dilute the first portion with equal volume of water, add a piece of clean white adsorbent cotton to it and keep it overnight. If after washing gently in water the cotton piece continues to retain straw colour which turns pink by a drop of stannous chloride solution, it confirms the presence of annatto. To the second portion add boric acid solution to make the solution acidic. If red colour appears, turmeric is present and if pink colour appears it indicates the presence of curcumin, sometimes turmeric and curcumin are not eluted if present in small amount. Notice the change of colour of the column for their detection.

22.4.5.2 *Detection of carotene*

To the non-alkali treated portion add saturated solution of antimony trichloride in chloroform. If blue colour appears, carotene is present.

23 ANISIDINE VALUE

23.1 Principle

A test solution is prepared in isooctane (2, 2, 4-trimethylpentane). It is reacted with an acetic acid solution of *p*-anisidine. The increase in absorbance at 350 nm is measured. The anisidine value is calculated.

23.2 Reagents

23.2.1 *Sodium sulfate (Na_2SO_4), anhydrou*

23.2.2 *Isooctane (2, 2, 4-trimethylpentane),* having an absorbance not exceeding 0.01 against water in the wavelength range 300 nm to 380 nm.

23.2.3 *4-Methoxyaniline (p-anisidine),* anhydrous cream-coloured crystals.

WARNING — *p*-anisidine is toxic and care shall be taken to avoid contact with the skin.

Store the *p*-anisidine in a dark bottle at 0 °C to 4 °C in the dark.

No coloration (grey or pink) shall be observed. If this is present, purify the *p*-anisidine as follows.

Dissolve 4 g of *p*-anisidine in 100 ml of water at 75 °C. Add 0.5 g of sodium sulfite (Na_2SO_3) and 2 g of charcoal. Stir for 5 min and filter through a medium retention filter paper to give a clear solution. Cool the filtrate to 0 °C and leave at this temperature for at least 4 h. Filter off the crystals, preferably under vacuum, and wash with a small volume of water at about 0 °C. Dry in a vacuum desiccator containing an efficient desiccant.

23.2.4 Glacial acetic acid, of water content not greater than 0.1 percent (mass fraction).

23.2.5 *Anisidine Reagent* — On the day of use, prepare the minimum quantity of reagent required for the analysis, in view of its toxicity and limited life. Prepare, for example, 50 ml of reagent as follows.

Dissolve 0.125 g of the *p*-anisidine (23.2.3) in the glacial acetic acid (23.2.4) in a 50 ml volumetric flask and dilute to the mark with the same solvent, avoiding exposure to strong light.

Check the absorbance against isooctane before use and discard the reagent when the difference is larger than 0.2. In any case, discard any reagent left over on the day of use.

23.3 Apparatus

Usual laboratory apparatus and, in particular, the following:

23.3.1 *Spectrometer*, double- or single-beam, suitable for use at a wavelength of 350 nm, with cells of optical path length 10 mm. When a double-beam spectrometer is used, it is recommended that a pair of matched 10 mm cells be used.

23.3.2 *Volumetric Fasks,* of 25 ml capacity.

23.3.3 *Test Tubes,* of 10 ml capacity, fitted with ground glass stoppers.

23.3.4 *Pipettes,* of 1 ml and 5 ml capacities, equipped with a safety suction device.

23.4 Procedure

23.4.1 *Test Portion and Preparation of Test Solution*

Weigh, to the nearest 1 mg, a sufficient mass of the prepared test sample directly into a 25 ml volumetric flask. Preheat solid samples to 10 °C above their melting point. Dissolve the sample in 5 ml to 10 ml of the isooctane and make up to the mark with the same solvent. The size of the test portion depends on the quality of the sample and the characteristics of the spectrometer used, and should be chosen to avoid readings near the upper and lower ends of the scale. In general, 0.4 g to 4.0 g is used.

23.4.2 *Unreacted Test Solution*

By means of a pipette, transfer 5 ml of the test solution to a test tube. Add 1 ml of glacial acetic acid, stopper the tube and shake well. Keep the test tube in the dark at (23 ± 3) °C for 8 min. Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of 10 min ± 1 min, follow the procedure specified in 23.4.5.

23.4.3 *Reacted Test Solution*

Transfer, by means of a pipette, 5 ml of the test solution to a test tube. Add, by means of a pipette, 1 ml of the anisidine reagent (*see* **23.2.5**). Stopper the tube and shake well. Keep the test tube in the dark at (23 ± 3) °C for 8 min. Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of 10 min ± 1 min from the addition of the anisidine reagent, follow the procedure specified in **23.4.5.**

23.4.4 *Blank*

Transfer, by means of a pipette, 5 ml of isooctane to a test tube. Add, by means of a pipette, 1 ml of the anisidine reagent (*see* **23.2.5**). Stopper the tube and shake well. Keep the test tube in the dark at (23 ± 3) °C for 8 min. Within a further 2 min, transfer the solutions to a clean, dry spectrometer cell. After a total reaction time of 10 min ± 1 min from the addition of the anisidine reagent, follow the procedure specified in **23.4.5**.

23.4.5 *Spectrometric Measurement*

Adjust the zero absorption of the spectrometer with isooctane at 350 nm. Measure the following absorbances against isooctane:

a) A_0 of the unreacted test solution (*see* **23.4.2**),

b) A_1 of the reacted solution (*see* **23.4.3**), and

c) A_2 of the blank (*see* **23.4.4**).

23.4.6 *Absorbance Range*

If the measured absorbance A_1 of the reacted solution (23.4.3) is not in the range 0.2 to 0.8, repeat the determination (*see* **23.4.2** to **23.4.4**) with an adjusted amount of test sample.

If the measured absorbance A_2 of the blank exceeds 0.2, purify the anisidine reagent as described in **23.2.3**, and prepare fresh anisidine reagent (**23.2.5**). Repeat this test with the fresh anisidine reagent.

23.5 Calculation

23.5.1 The anisidine value (AV) of the sample is equal to the formula given below:

$$AV = \frac{100\ QV}{m} [1, 2\ (A_1 - A_2 - A_0)]$$

where

V = volume in which the test sample is dissolved, in millilitres (V = 25 ml);

m = mass of the test portion, in grams;

Q = sample content of the measured solution based on which the anisidine value is expressed, in grams per millilitre (Q = 0.01 g/ml);

A_0 = absorbance of the unreacted test solution (*see* **23.4.2**);

A_1 = absorbance of the reacted solution (*see* **23.4.3**);

A_2 = absorbance of the blank (*see* **23.4.4**); and

1.2 = correction factor for the dilution of the test solution with 1 ml of the reagent or glacial acetic acid.

NOTE — Report the results to 1 decimal place.

23.5.2 When assessing the oxidative deterioration of an oil, the total oxidation value, or 'totox value' (TV), may be helpful. The calculation is shown in the formula given below [with the peroxide value (PV) expressed in meq O_2/kg]:

$$TV = (2 \times PV) + AV$$

24 CLOUD POINT IN PALMOLEIN

24.1 Apparatus

24.1.1 *Oil Sample Bottle*, 115 ml (4 oz).

24.1.2 *Thermometer,* range 2 °C – 68°C.

24.1.3 *Water Bath,* made up of water, chipped ice and water or chipped ice, salt and water, depending upon the temperature required. The temperature of the water bath shall not be less than 2 °C and not more than 5 °C of the cloud point.

24.2 Procedure

The sample must be completely dry before making the test. Heat 60 to 75 g of sample to 130 °C just before the test. Pour ca 45 ml of the heated fat into an oil sample bottle. Place the bottle in a water bath. Begin to cool the bottle in the water bath, stirring enough using the thermometer to keep the temperature uniform. When the sample has reached a temperature ca 10 °C above the cloud point, begin stirring steadily and rapidly in a circular motion so as to prevent super-cooling and solidification of fat crystals on the sides or bottom of the bottle. From this point on, do not remove the thermometer from the sample, since to do so may introduce air bubbles which will interfere with the test. Maintain the test bottle in such a position that the upper levels of the sample in the bottle and the water in the bath are about the same. Remove the bottle from the bath and read the temperature. The bottle should be inspected regularly. The cloud point is that temperature at which that portion of the thermometer immersed in the oil is no longer visible when viewed horizontally through the bottle.

NOTE — This test is useful for the detection of palmolein in groundnut oil. Presence of palmolein over 10 per cent in groundnut oil readily gives cloud at a higher temperature than that of groundnut oil due to the presence of palmitic glycerides in higher amounts in palmolein/palm oil.

25 DIENES AND TRIENES

25.1 Principle

Oxidised fatty acids containing conjugated double bonds absorb UV strongly between 230 and 375 nm, dienes absorbing at 234 nm and trienes at 268 nm. Conjugated trienes may be formed by industrial processing, for example, decolorising with bleaching earths. A secondary absorption by trienes occurs at about 278 nm. In the early stages of oxidation the UV absorption increases somewhat proportionately to the uptake of oxygen and the formation of peroxides. The UV absorption curve forms plateau just before the end of the induction period. The magnitude of UV absorbance is not readily related to the amount of oxidation; so the method is best applicable to detecting relative changes in oxidation of an oil in comparison experiments or stability tests.

25.2 Procedure

Weigh accurately into a 25 ml volumetric flask, an amount of the oil sample so that the absorbance of its solution in iso-octane in a 10 mm quartz cell lies between 0.2 and 0.8. Trace the absorption curve against iso-octane between 220 and 320 nm and select the wavelength 1 max of maximum absorption near 230, 268 and 278 nm, and the absorbance (A) at these points.

Specific absorbance $E_{1cm}^{1\%}\ (\lambda_{max}) = A/(c \times d)$

where

c = concentration of the sample solution (g/100 ml); and

d = cell length in cm.

26 ALKALINITY

26.0 Applicability

This method determines alkalinity of animal and vegetable fats and oils without distinguishing between the various constituents. The method is not applicable to dry melted animal fats, nor to oils and fats with an acidity greater than 60 percent (mass fraction).

NOTE — Fats and oils can contain alkaline constituents either naturally (for example, calcium soaps from bones) or accidentally (for example, sodium soaps in imperfectly refined fats and oils).

26.1 Principle

A test portion is dissolved in warm aqueous acetone and titrated with hydrochloric acid.

26.2 Reagents

26.2.1 *Distilled Water*

26.2.2 *Aqueous Acetone*

Mix 970 ml of acetone, 20 ml of water and 10 ml of bromophenol blue solution (2 g/l in acetone). Add sodium hydroxide solution [c (NaOH) = 0.1 mol/l] to give a blue colour, then add hydrochloric acid [c (HCl) = 0.1 mol/l] until a yellowish green colour appears.

26.2.3 Hydrochloric acid standard volumetric solution, c (HCl) = 0.01 mol/l, standardized not more than 7 days before use.

26.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

26.3.1 *Borosilicate Glass Apparatus*, for aqueous acetone (**26.2.2**).

26.3.2 *Conical Flasks,* with wide necks, and of capacities 400 ml to 500 ml.

26.4 Procedure

26.4.1 *Preparation of the Apparatus*

Rinse each conical flask **(26.3.2**) and stirrer to be used with successive 20 ml amounts of aqueous acetone (**26.2.2**) until the aqueous acetone does not change colour. Allow the flasks and stirrer to dry.

26.4.2 *Test Portion*

From the test sample, weigh, to the nearest 0.1 g, a test portion of up to 40 g (see below Table 10) according to the expected result and colour of the sample. Melt a solid test portion at below the boiling point of acetone. Add 100 ml of the acetone (26.2.2) at 40 °C and stir. Allow to settle until the solution splits in two layers; in the presence of soap, the upper layer is blue-coloured.

For test portions of less than 10 g, take replicate portions totalling at least 5 g for titration and sum the masses and titres to give the result for one determination.

26.4.3 *Determination*

Titrate the warm (30 °C to 40 °C) solution with the hydrochloric acid (**26.2.3**) while stirring until the colour of the indicator changes from blue (or blue green) to the yellowish green of the acetone (**26.2.2**) added. Allow the mixture to settle in order to observe the colour clearly.

NOTE — An explosion-resistant hotplate with magnetic stirrer and burette support is particularly useful.

26.5 Calculation

Alkalinity may be expressed as a mass, in milligrams, of sodium oleate per kilogram of fat or oil, or as a percentage (mass fraction) of sodium hydroxide:

a) the alkalinity in terms of sodium hydroxide is given by the expression:

$$A = \frac{4\,Vc}{m}$$

b) the alkalinity in terms of sodium oleate is given by the expression:

$$S = \frac{V \times \mathrm{c} \times 304400}{\mathrm{m}}$$

where

A = alkalinity expressed as a percentage (mass fraction) of sodium hydroxide;

S = alkalinity expressed as milligrams of sodium oleate per kilogram of sample;

m = mass of the test portion, in grams;

c = exact concentration of the standard volumetric hydrochloric acid solution used, in moles per litre; and

V = volume of hydrochloric acid standard volumetric solution used to titrate the test portion, in millilitres.

Table 10 Mass of Test Portion

(*Clause* 26.4.2)

Colour of sample	Expected result	Mass
	mg sodium oleate/kg of sample	g
Light	up to 500	40
Light	over 500 to 1 000	10
Light	over 1 000	4
Dark	not relevant	2
Very dark	not relevant	1

ANNEX A

(*Foreword*)

COMMITTEE COMPOSITION

Oils and Oilseeds Sectional Committee, FAD 13

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